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ERRATA TO VOLUME II.

Page 39, line 1, *for* " f=values " *read* " f=values " and *for* " t-values " *read* " t-values ".

Page 40, line 2, *for* " t=formula " *read* " t-formula " .

Page 50, line 7 from bottom, *for* " aud " *read* " and ".

Page 57, Table I, heading of last column, *for* " loge " *read* " $\log_e$ ".

Page 67, Table II, column 1, line 1, *for* ' Co_2 ' *read* " CO_2 ".

Page 122, legend of Table XXI, and heading of column 2, *for* " NH''_4 " *read* " NH'_4".

Page 142, Table I, column ' H ', 1st row, *for* " 1×10^{7} " *read* " 1×10^{-7} ".

Page 143, Table II, last but one column, 2nd row, *for* " 1×10^{6} " *read* " 1×10^{-6} ".

Page 154, Table VI, heading of Column 2, *for* " 1×10^{5} " *read* " 1×10^{-5} ".

Page 154, Table VI, heading of Column 4, *for* " 1×10^{7} " *read* " 1×10^{-7} ".

Page 154, Table VI, heading of Column 5, *for* " 1×10^{8} " *read* " 1×10^{-8} ".

Page 154, Table VI, last column, last but one row *for* " $\frac{1}{8}$ " *read* " $\frac{1}{8}$ ".

Page 231, line 12, *for* " phenophthalein " *read* " phenolphthalein ".

Page 232, heading of Table II, *for* " *Titratian* " *read* " *Titration* ".

Page 249, Table, sub-head 2, *for* " Adualts " *read* " Adults ".

Page 261, Table IV continued, heading of column 2, *for* " contractor " *read* " character ".

Page 267, line 17 from below, *for* " fiig " *read* ' fig '.

Page 267, line 13 from below, *for* " ALBIONS " *read* " ALBINOS ".

Page 287, line last but one, *for* " in " *read* " is ".

Page 291, line 9 from below *for* " existance " *read* " existence ".

Page 429, line 11, *for* " irrepective " *read* " irrespective ".

Contents page of Part V, line 9 from bottom *for* " (*& g.*) " *read* " (*Ag.*) ".

Page 454, line 14 from bottom, *for* " Sawyer, A. J., A. M." *read* " Sawyer, A. M.".

Page 458, Table III, right hand column heading, *for* " ovenn-dry " *read* " oven-dry ".

Page 501, line 6, *for* " 70° 80°F." *read* " 70°-80°F.".

Page 501, line 14, *for* " sightly " *read* " slightly ".

Page 503, Table I, column 2, row 2, *for* " *Phyllosticta rabiel* " *read* " *Phyllosticta rabiei* ".

Page 529, line 11 from bottom *delete* " comma " *after* " would ".

Page 540, line 15, *for* " *Enio* " *read* " *Ento* ".

Page 569, line 4 from bottom, *for* " *Velucelles* " *read* " *Volucelles* ".

Page 570, line 9, *for* " Mame " *read* " Maine ".

Page 642, line 8, *for* " 35 ± 1°C." *read* " $35\pm\cdot1$° C.".

Page 644, footnote, line 2, *for* " Ago/AgCl " *read* " Ag/AgCl ".

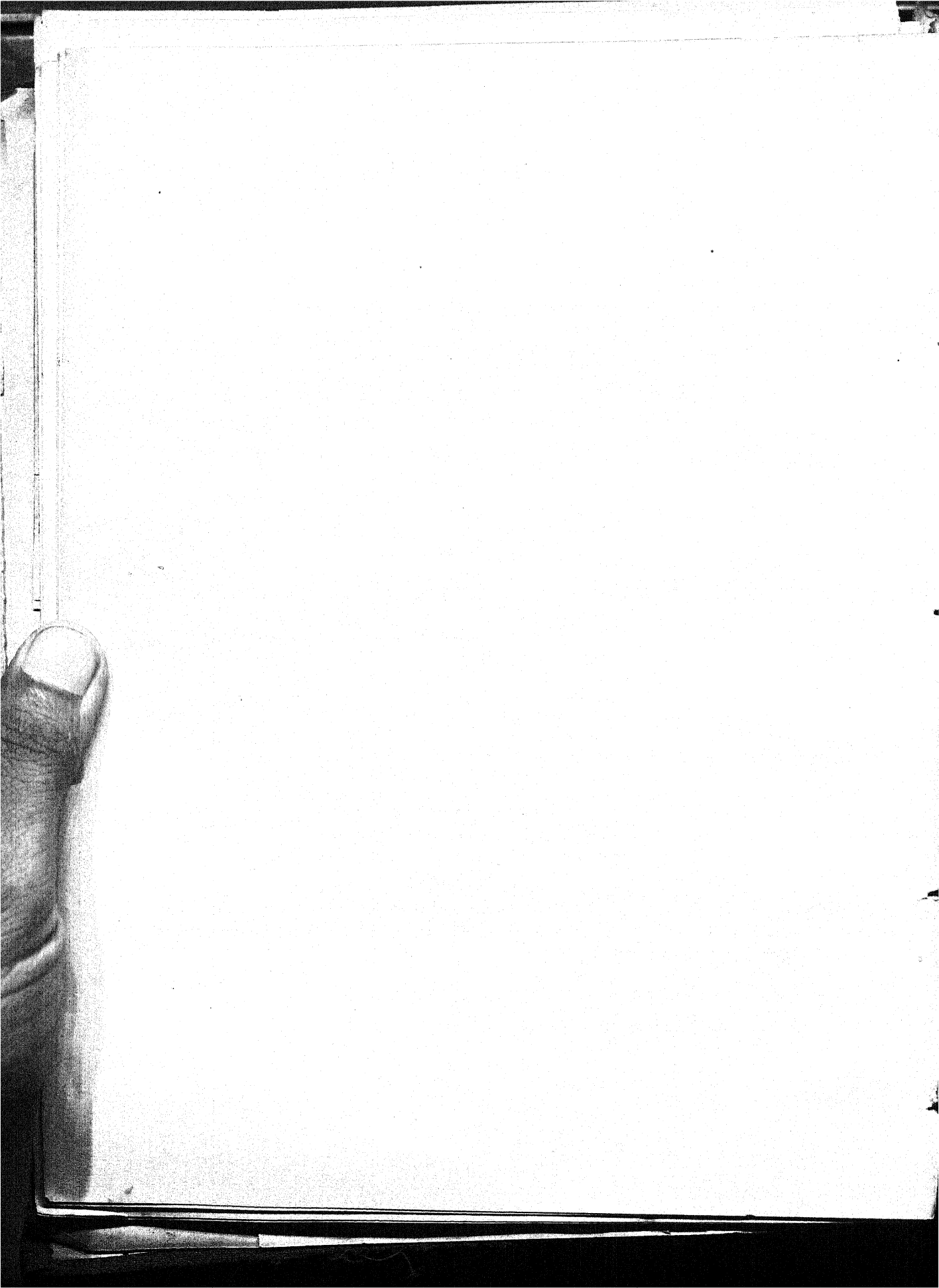
Page 654, line 6 after Table V, *for* " siticic acid " *read* " silicic acid ".

Page 679, line 12, *for* " loge " *read* " $\log_e$ ".

Pages 683, 684, and 685 Heading of Tables I and II *for* " $2z^1$ " *read* " 2 z' ".

Page 714, 5th line from bottom, *for* " They are sent free within the British Empire, a charge of 4*s*. per annum is made " *read* " They are sent free, within the British Empire, to all recipients of publications on Soils and Fertilizers. Outside the British Empire, a charge of 4*s*. per annum is made ".

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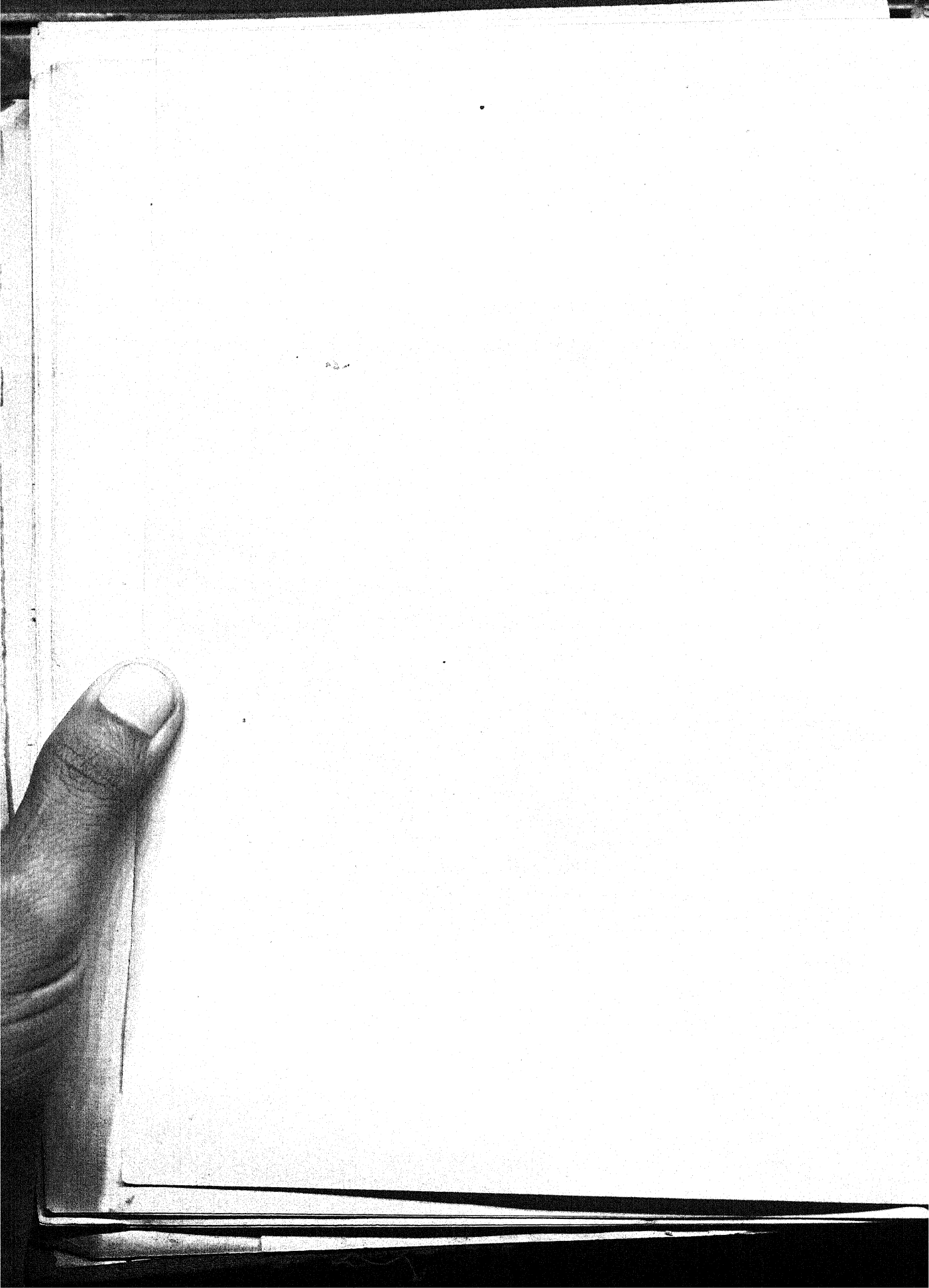
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ORIGINAL ARTICLES

A STUDY OF THE PHYSICO-CHEMICAL CHANGES ACCOMPANYING THE PROCESS OF RECLAMATION IN ALKALI SOILS.

BY

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(Received for publication on the 8th September 1931.)

(With Plate I and two text-figures.)

Introduction.

The problem of reclaiming alkali and *kallar* soils is as old as the art of agriculture. The presence of alkali in the soils and the methods of their reclamation were studied long before the origin or nature of *kallar* soils was understood by the scientists. The earlier workers attributed the toxicity of alkali soils exclusively to the presence of excess of soluble salts, and the general opinion then held was, that these toxic conditions could be removed by leaching with water. This method only proved to be palliative in certain cases only, and no permanent cure was effected. Later on Berthollet, Hilgard and Mondesir pointed out, that under certain conditions these excessively soluble salts, especially those of sodium, caused the formation of sodium carbonate, which had a harmful effect on the plant roots. Since the toxic condition was believed to be due to the presence of free alkali, methods were sought by which alkalinity could be neutralised with the help of an acid. Lipman [1916] suggested the use of sulphuric acid and elemental sulphur for this purpose. P. L. Hibbard [1921], working on the suggestion of Lipman, first tried the effect of sulphur coupled with leaching, but later on he [1922] studied the effect of gypsum as a possible means of supplying the sulphate radical.

The discovery of gypsum and its fertilising value was made known as early as the latter part of the eighteenth century, but how it acted was at the time little understood. Hibbard as the result of his experiments with gypsum concluded that excessive alkalinity could be neutralised with gypsum, but as the reaction by

which sodium carbonate is converted into sodium sulphate is a reversible one, there is every tendency of the back-reaction setting in. The only method of preventing the reversal of this reaction is either by increasing the concentration of calcium sulphate in the soil solution or by removing the products of reaction through leaching. The former is impracticable on account of the low solubility of gypsum in water, and the latter has been practised with a certain amount of success.

All these methods were being practised with varying success on different types of *kallar* soils when the researches of Gedroiz, Hissink, and de' Sigmond on the phenomenon of base-exchange in soils were rendered available. These scientists working independently in different countries established an important fact that the soils possess a remarkable property of base-exchange. On the basis of this theory they pointed out that a portion of the soil base which usually resides in the clay fraction is always held up in the soil in a different condition from the rest of the bases, and other things being equal, is directly responsible for the fertility of the soil.

The phenomenon of base-exchange as studied by these authors furnished a basis for further investigations into the nature and formation of *kallar* soils. Joffe and Mclean [1924, 1, 2, 3, 4] took up Gedroiz's idea and studied the colloidal, physical, chemical and biological aspects of alkali soils. Kelley and Brown [1925] made a detailed study of alkali soils with special reference to base-exchange phenomenon, and came to conclusions more or less similar to those arrived at by Gedroiz and Hissink. All these base-exchange workers definitely proved that a normal soil consists mainly of calcium clay, but under adverse conditions excess of sodium salts can react with the clay fraction and produce what may be designated as sodium clay. This sodium clay when once produced cannot be cured by methods of leaching alone. For any permanent cure the first essential is to break up sodium clay with the help of chemicals such as gypsum or calcium chloride which would re-convert sodium clay into calcium clay.

From the practical point of view it is important to know what fraction of the total exchangeable sodium in alkali soil could be replaced by calcium before a crop can be grown on it successfully. It is also necessary to know what amount of these chemicals would be needed to bring about this change in the soil under field conditions. The two important sources of calcium for this purpose are gypsum and calcium chloride. The former suffers from a little disadvantage on account of its being sparingly soluble in water. The exchange reaction, as already pointed out, is a reversible one and proceeds according to the following equation :—

$$\text{Sodium clay} + CaSO_4 \rightleftharpoons \text{Calcium clay} + Na_2SO_4$$

Even if the products of reaction are thoroughly leached, it is extremely difficult to bring the reaction to completion owing to the limited solubility of calcium

sulphate in water. In this respect calcium chloride possesses a distinct advantage over gypsum. If this reaction were to proceed according to the above equation, in that case with the help of calcium chloride, it should be possible to complete the reaction with one application alone. Thus, calcium chloride presents a possibility of reclaiming alkali soils with one heavy dose of the chemical to be followed by thorough leaching with water.

The physico-chemical changes in the soil accompanying the course of replacement of sodium by calcium with the help of these chemicals are little understood. The object of the present investigation was to study these changes in detail during the entire process of reclamation on representative types of *kallar* soils in the province.

Experimental.

Four different types of *kallar* soils, one each at Lyallpur, Kala Shah Kaku, Montgomery and Bara Farm, were selected for the experiment, As pointed out by Kelley [1922] alkali soils are exceedingly variable in composition, therefore, considerable difficulty was experienced in selecting a piece of land uniformly affected with *kallar*. Broadly speaking, from the *kallar* point of view the area at Lyallpur has a very high concentration of salts, and Kala Shah Kaku soil represents typical rice land, while the soil at Montgomery is of *bari* nature, and in respect of the alkali trouble is believed to be the worst of the whole lot. Experiments were conducted on all the different types of *kallar* soils, but in the present communication we will merely confine ourselves to observations made on *bari* type of soil and reserve the remaining data for future consideration.

Nature of soil.

Amongst the alkali soils in the Province, the Montgomery soil ordinarily known as *bari* is a fairly common type, and is chiefly to be found in the semi-arid districts of Montgomery, Multan, and Lyallpur. No vegetation of any description whatsoever grows on these lands and from a distance the whole area represents the appearance of a mirage. The surface soil is extremely hard, difficult to plough and lacking in permeability. On irrigation, water remains on the surface for a number of days and very little of it percolates into the sub-soil. On drying, the soil cracks into hexagonal shaped blocks and forms a crust on the surface known as 'Papri'. The soil contains a fair amount of calcium carbonate and is more or less uniformly impregnated with *kallar* salts, having a maximum concentration at a depth of 4 ft. The soluble salts vary in the top layer from 0·63-1·07 per cent., and the mean figure for a four feet column is about 1 per cent. Mechanical analyses of two represen-

tative samples of *bari* soil and the 'Papri', usually found on the surface of such soils gave the following figures :—

TABLE I.

—	Clay	Fine silt	Silt	Fine sand	Sand	Gravel
Montgomery soil . . .	19·01	14·26	20·12	45·28	0·51	..
	20·69	13·45	24·12	38·60	2·20	..
Papri	78·40	13·00	3·28	..	..	..

Merely on the basis of mechanical texture, the soil ought to manifest average fertility, but experience shows that such is not the case. Again, when the results of chemical analysis (Table II) are examined, it is found that the soil possesses a fair percentage of potash and phosphate but is somewhat lacking in nitrogen and organic matter. The loss of organic matter usually occurs in alkali soils on leaching, as a result of which valuable organic substances are lost owing to the solubility and high dispersion of these substances in alkaline solutions.

TABLE II.

—	Chemical analysis	Available analysis
Insoluble residue	78·98	...
	79·85	
Soluble silica	0·20	...
	0·19	
Iron as Fe_2O_3	4·13	...
	4·39	
Aluminium as Al_2O_3	5·79	...
	5·80	
Calcium as CaO	3·86	...
	3·82	
Magnesium as MgO	2·18	...
	2·41	
Sodium as Na_2O	0·5822	..
	0·5419	

TABLE II—*contd.*

	Chemical analysis	Available analysis
Potassium as K_2O	0·6612	0·0885
	0·8523	0·1017
Phosphorus as P_2O_5	0·2482	0·0948
	0·2631	0·0911
Nitrogen as N	0·0294	...
	0·0238	...
Organic matter	0·39	...
	0·30	...

From the point of view of soluble salts it was observed that, even though by leaching the total solids are reduced considerably, the soil itself does not improve. Consequently there is something radically wrong with the soil which is responsible for its complete infertility. As we shall show later on, the *kallar* trouble in these soils is mainly due to a deficiency in exchangeable calcium, and can be explained in terms of the ratio between monovalent and divalent bases present in these soils.

Plan of experiment.

Plots measuring 1/20 of an acre were laid out according to the following scheme :—

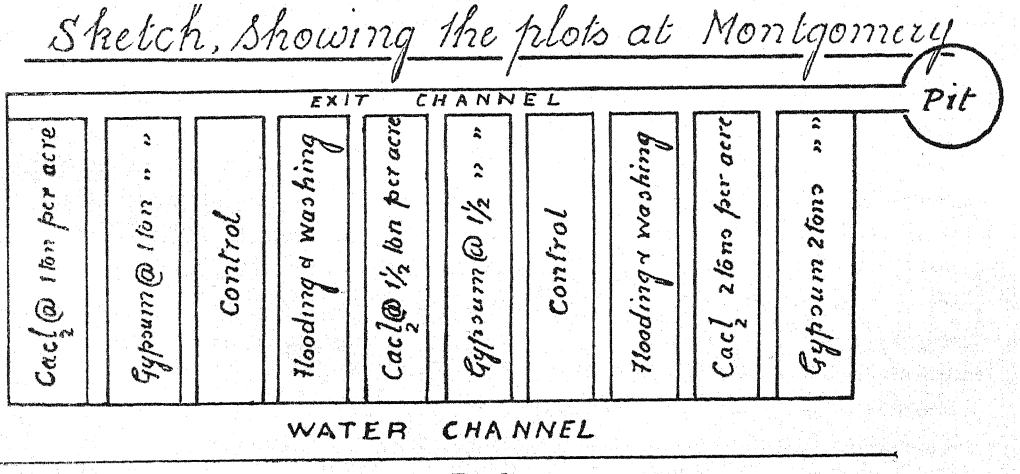


FIG. 1.

The whole area measuring 4 *kanals* is divided into 10 plots with a number of controls, and with an interstrip and a double bund between each plot. The object of the double bund is to prevent overflowing of water from one plot to the other. Alongside these plots exists an exit channel which carries the drainage water into a pit.

Treatment.

All the plots were first thoroughly soaked and leached with water. Then powdered calcium chloride and gypsum at the rate of 1, 1½, and 2 tons per acre were ploughed into the soil. In this condition the plots were left over for about four months during which period they were occasionally watered and stirred up in order to ensure thorough mixing up of the chemicals with the soil. At the end of this period all the plots were leached with water again, and a suitable crop was sown in them. A similar treatment was followed for another three years. At the end of the fourth year, farmyard manure at the rate of 14 tons per acre was applied to all the experimental plots as it was observed that although they had improved their lime status, they were still deficient in certain essential plant-food materials.

Soil Sampling.

Soil sampling was done in individual plots before and after each treatment. With the help of a soil auger one foot samples were obtained down to a depth of 4 ft. While sampling after the treatment, it was observed that the soils in the treated plots had become distinctly porous, and moisture in them had travelled to a greater extent than in controls or leached plots.

A reference to the moisture curves reproduced below shows the extent to which moisture has travelled in different plots. Calcium chloride plots show on the whole the highest moisture content, followed by gypsum, leached and control plots. In the 9th plot which has received calcium chloride at the rate of 2 tons per acre the amount of moisture even at a depth of 6 feet is about 17 per cent., whereas in plots VII and VIII (control and leached plots) the moisture content at the same depth is about 6-7 per cent. This clearly indicates the effect of these chemicals on the permeability of these soils. There is, however, a sudden break in the calcium chloride curves at a depth of 4 ft. while in other cases at a depth of 3 ft. This is due to a difference in the texture of the sub-soil strata which become

distinctly heavy and full of *kankar* between the depths of 3rd and 4th ft. Beyond this the curves follow a natural course.

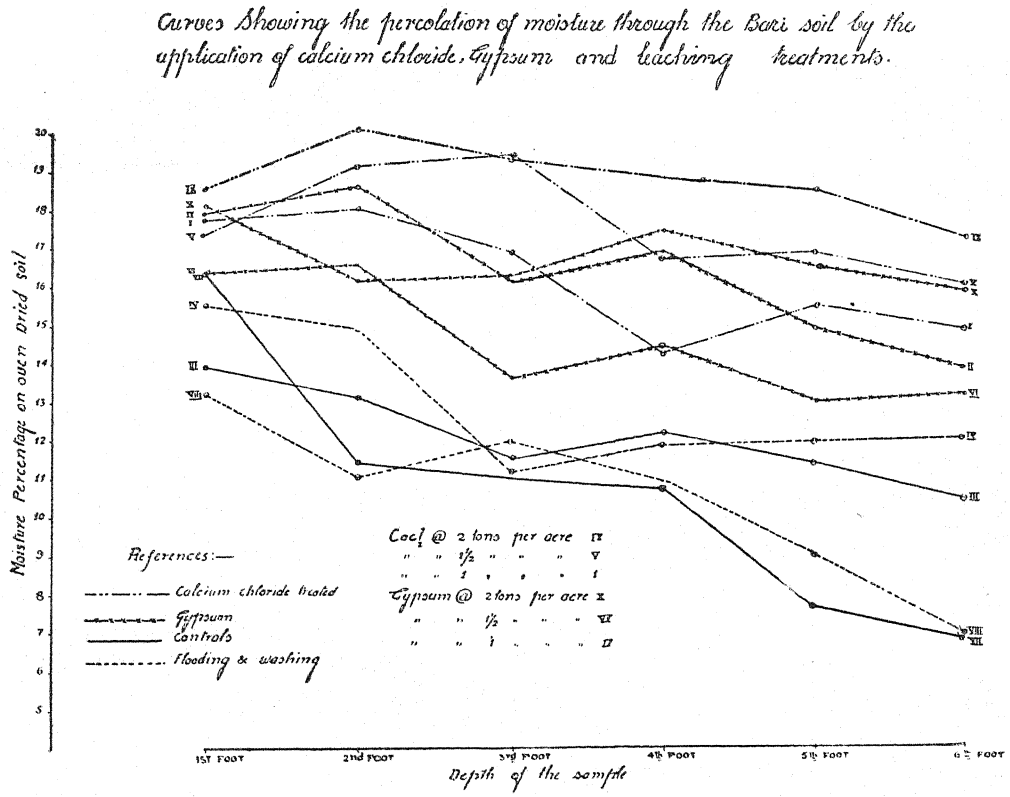


FIG. 2.

Crop.

Swank was sown in all the experimental plots during the first two years, but later on, it was substituted by wheat. The sowing of *swank* was done in the first instance because experience had shown that this is by far the best crop that can be

grown on these inferior soils with any measure of success. A statement of yields obtained from different plots during four successive years is given in Table III.

TABLE III.

Showing the crop yields at Montgomery Agricultural Station.

Plot No.	1927-28				1928-29				1929-30				1930-31			
	Swank				Swank				Wheat				Wheat			
	Yield per Acre				Yield per Acre				Yield per Acre				Yield per Acre			
	Grain			Straw	Grain			Straw	Grain			Straw	Grain			Straw
	Mds.	Srs.	Ch.	Mds. Srs. Ch.	Mds.	Srs.	Ch.	Mds. Srs. Ch.	Mds.	Srs.	Ch.	Mds. Srs. Ch.	Mds.	Srs.	Ch.	Mds. Srs. Ch.
1	4	25	0	35 15 0	0	36	14	20 13 2	4	10	0	12 13 5	12	5	0	20 0 0
2	1	20	0	24 30 0	1	35	10	24 34 6	3	30	0	7 27 8	10	20	0	15 13 0
3		*Nil*		3 30 0	0	6	14	12 23 2	0	22	8	2 0 0	1	30	0	2 20 0
4	0	0	10	4 39 6	0	16	14	11 33 2	1	0	0	4 7 8	4	0	0	6 0 0
5	1	27	8	28 12 8	2	1	4	20 8 12	5	30	0	12 30 0	14	25	0	22 37 0
6	0	2	8	2 7 8	0	30	0	10 20 0	2	0	0	5 25 0	5	23	0	8 20 0
7		*Nil*		*Nil*		*Nil*		*Nil*		*Nil*		*Nil*		*Nil*		*Nil*
8		*Nil*		*Nil*		*Nil*		*Nil*		*Nil*		*Nil*		*Nil*		*Nil*
9	0	15	0	13 15 0	1	31	14	19 18 2	3	20	0	8 20 0	9	13	0	14 27 0
10	0	8	2	7 11 14	0	10	10	19 29 6	2	30	0	5 0 0	3	10	0	5 3 0

A reference to this table shows a progressive improvement of the treated plots from year to year. The maximum improvement, however, has been noticed in the 4th year when in addition to the ordinary treatment an average dose of farmyard manure was also administered. During that year the calcium chloride treated plots have given about the same yield as can be expected from a normal soil in that locality. The gypsum-treated plots are next in order, while there is hardly any difference between controls and the leached plots both giving hardly any yield. Inspite of the fact that plots IX and X received the highest dose of treatment, yet they do not give the maximum yield. This is due to the fact that in the initial stages plots VI—X are distinctly very much inferior to plots I—V.

Exchangeable bases.

The estimation of the exchangeable bases, *viz.*, Ca, Mg, Na and K of the first foot soil samples, taken before and after each treatment, was done according to the method of Hissink [1923]. While carrying out this method it was observed that,

though it is generally applicable to alkali soils, yet certain difficulties are experienced in case of soils containing either sodium carbonate or water-soluble calcium. However, both these are practically absent in Montgomery soils. Kelley's suggestion [1930], regarding the use of hot sodium chloride solution for treating the soil in order to give a uniform extraction of $CaCO_3$, in both the litres of the filtrate, was already being followed as a modification in Hissink's method.

A statement of exchangeable calcium content of these soils, given in Table IV, shows that each application of the chemicals improves the lime status of the soil.

TABLE IV.

Exchangeable calcium depicted as percentage on air-dried soil (before and after treatment).

Plot No.	1st treatment		2nd treatment		3rd treatment		4th treatment	
	Before	After	Before	After	Before	After	Before	After
1	0·0496	0·0556	0·0586	0·0651	0·0674	0·0845	0·0719	0·0905
2	0·0487	0·0531	0·0472	0·0716	0·0657	0·0724	0·0630	0·0711
3	0·0583	0·0424	0·0570	0·0358	0·0573	0·0539	0·0500	0·0436
4	0·0403	0·0403	0·0504	0·0371	0·0606	0·0320	0·0420	0·0436
5	0·0445	0·0445	0·0586	0·0699	0·0859	0·0892	0·0921	0·0872
6	0·0169	0·0339	0·0438	0·0387	0·0758	0·0842	0·0646	0·0824
7	0·0212	0·0249	0·0215	0·0260	0·0337	0·0269	0·0275	0·0242
8	0·0416	0·0294	0·0437	0·0263	0·0286	0·0320	0·0275	0·0162
9	0·0395	0·0665	0·0707	0·0879	0·0774	0·1014	0·0824	0·1164
10	0·0295	0·0499	0·0569	0·0731	0·0758	0·0926	0·1164	0·1261

Treatment with chemicals was continued for a period of four years on the assumption that a normal soil contains about 0·1 per cent. of exchangeable calcium. Another fact which led to the same belief is furnished by experiments (described elsewhere) on *bari* soil in percolation tubes, which showed that when maximum replacement of sodium by calcium takes place, the calcium content of this type of soil is improved from 0·03 to 0·14 per cent. Thus in all these experiments an attempt was made to raise the content of exchangeable calcium to about 0·1 per cent. through successive treatments.

Table V indicates the extent to which sodium can be replaced by calcium in the exchange complex over a period of 4 years. A study of this table reveals that an

application of calcium chloride and gypsum at the rate of 1 ton per acre replaces sodium to a limited extent only, and the lime status of the plots is improved from 19 to 49 in one case, and 22 to 49 in the other. Beyond this, however, there is no more replacement with further treatments of the chemicals. This may be due to the fact that at that particular stage an application of 1 ton per acre of the chemicals does not produce sufficient concentration of calcium in the soil solution to bring about further displacement of sodium by calcium. A maximum replacement, however, in these experiments is effected through a treatment of 2 tons per acre, when calcium is improved from 11 to 89 in one case, and 10 to 72 in the other. These results clearly point out the possibility of greater replacement of sodium by calcium with heavy doses of the chemicals given in few instalments. The calcium-sodium ratio as depicted in the first column (before any treatment) also shows the relative fertility of the plots before the experiments were undertaken. Plots I—V with a calcium content varying between 19 and 22 are distinctly superior to plots VI—X, with a calcium content varying between 10 and 11 only.

TABLE V.

Ratio of calcium to sodium depicted as percentage on air-dried soil.

(Before and after treatment.)

Plot No.	1st treatment		2nd treatment		3rd treatment		4th treatment	
	Before	After	Before	After	Before	After	Before	After
1	19 : 81	35 : 65	41 : 59	49 : 51	44 : 56	48 : 52	46 : 54	46 : 54
2	22 : 78	29 : 71	43 : 57	44 : 56	43 : 57	46 : 54	44 : 56	43 : 57
3	20 : 80	23 : 77	36 : 64	21 : 79	23 : 77	22 : 78	19 : 81	17 : 83
4	21 : 79	23 : 77	32 : 68	21 : 79	24 : 76	22 : 78	20 : 80	20 : 80
5	20 : 80	28 : 72	37 : 63	45 : 55	53 : 47	53 : 47	52 : 48	58 : 42
6	11 : 89	20 : 80	28 : 72	24 : 76	48 : 52	50 : 50	42 : 58	51 : 49
7	11 : 89	11 : 89	11 : 89	13 : 87	15 : 85	15 : 85	13 : 87	12 : 88
8	11 : 89	11 : 89	20 : 80	11 : 89	17 : 83	17 : 83	11 : 89	10 : 90
9	11 : 89	24 : 76	37 : 63	53 : 47	57 : 43	66 : 34	79 : 21	89 : 11
10	10 : 90	18 : 82	37 : 63	43 : 57	43 : 57	52 : 48	66 : 34	72 : 28

Table VI gives the ratios of monovalent to divalent bases before and after each treatment. This confirms the general conclusions already arrived at on the basis of calcium-sodium ratio. A higher ratio of divalent to monovalent bases contributes to the fertility of the soil, while a low ratio contributes to infertility. It also indicates the relative fertility of the plots before the experiments were undertaken. More definite results based on the ratios of monovalent and divalent bases have, however, been obtained from pot experiments to be described later.

TABLE VI.

Ratio of divalent to monovalent bases depicted as percentage on air-dried soil.

(Before and after treatment.)

Plot No.	1st treatment		2nd treatment		3rd treatment		4th treatment	
	Before	After	Before	After	Before	After	Before	After
1	20 : 80	38 : 62	36 : 64	44 : 56	47 : 53	48 : 52	44 : 56	45 : 55
2	21 : 79	32 : 68	39 : 61	41 : 59	40 : 60	44 : 56	41 : 59	41 : 59
3	20 : 80	21 : 79	36 : 64	28 : 72	25 : 75	26 : 74	23 : 77	23 : 77
4	20 : 80	22 : 78	32 : 68	24 : 76	25 : 75	26 : 74	22 : 78	22 : 78
5	19 : 81	26 : 74	36 : 64	43 : 57	49 : 51	50 : 50	50 : 50	56 : 44
6	15 : 85	19 : 81	32 : 68	30 : 70	49 : 51	47 : 53	41 : 59	48 : 52
7	13 : 87	13 : 87	18 : 82	20 : 80	21 : 79	22 : 78	20 : 80	18 : 82
8	13 : 87	13 : 87	27 : 73	18 : 82	22 : 78	21 : 79	18 : 82	17 : 83
9	13 : 87	24 : 76	36 : 64	48 : 52	51 : 49	55 : 45	63 : 37	75 : 25
10	12 : 88	20 : 80	37 : 63	41 : 59	38 : 62	45 : 55	56 : 44	63 : 37

Table VII gives the pH values of the soil samples obtained from the experimental plots. The determinations were made with the help of an antimony

electrode [Snyder, 1928] and the results were further checked by comparing them with those of buffer solutions and soils of known pH value. These results point out that in the treated plots there has been a steady fall in the pH value, but the controls and the leached plots have shown increased alkalinity at the end of the experiment. This is due to the fact that in the case of treated plots, owing to the replacement of Na by Ca, and subsequent leaching the pH value is reduced, but in the case of controls and leached plots the sodium zeolites have a tendency to undergo hydrolysis which in the presence of soluble sodium salts is considerably retarded. But on leaching, the concentration of sodium salts is considerably reduced, and this promotes the hydrolysis of zeolites, which in turn is manifested by an increase in the pH value of the soil. This clearly points out that on such soil a treatment with calcium salts is essential, and that mere leaching of these soils cannot bring about any permanent cure and may on the other hand do more harm than good in the long run.

TABLE VII.

Hydrogen-ion concentration determined in I : 2·5 ratio of soil to water.

(*Before and after treatment.*)

Plot No.	1st treatment		2nd treatment		3rd treatment		4th treatment	
	Before	After	Before	After	Before	After	Before	After
1	8·66	..	8·68	9·60	8·70	8·73	8·01	8·09
2	8·70	8·68	8·93	8·80	8·89	9·12	8·70	8·32
3	8·50	8·61	9·32	9·28	9·03	9·09	9·19	9·41
4	8·80	9·00	9·24	10·00	8·90	9·77	8·94	9·47
5	8·90	..	8·90	9·60	8·73	9·14	7·92	8·60
6	9·30	9·30	9·23	9·40	8·90	9·06	8·16	8·70
7	9·10	9·47	9.64	9·78	9·82	9·88	9·57	9·68
8	9·10	..	9·64	10·32	10·16	9·85	9·98	9·71
9	9·10	8·80	9·14	9·02	8·77	8·83	8·38	8·22
0	9·00	9·00	9·12	9·30	9·57	9·13	9·25	9·03

Correlation of crop yields with ratios of divalent to monovalent bases.

Table VIII depicts the relative fertility of the plots based on the crop yields on the one hand, and the ratios of calcium to sodium and divalent to monovalent

TABLE VIII.

Statement showing the relationship of crop yields to the ratio of calcium to sodium and divalent to monovalent bases.

1st treatment			2nd treatment			3rd treatment			4th treatment		
Total yield of crop	Ratio of calcium to sodium	Ratio of divalent to monovalent	Total yield of crop	Ratio of calcium to sodium	Ratio of divalent to monovalent	Total yield of crop	Ratio of calcium to sodium	Ratio of divalent to monovalent	Total yield of crop	Ratio of calcium to sodium	Ratio of divalent to monovalent
					GROUP A						
I	I	I	II	I	I	V	V	V	V	V	V
V	V	II	V	V	V	I	I	I	I	I	I
II	II	V	I	II	II	II	II	II	II	II	II
IV	III	IV	III	III	III	IV	IV	III	IV	IV	III
III	IV	III	IV	IV	IV	III	III	IV	III	III	IV
					GROUP B						
IX	IX	IX	IX	IX	IX	IX	IX	IX	IX	IX	IX
X	VI	X	X	X	X	VI	X	VI	VI	X	X
VI	X	VI	VI	VI	VI	X	VI	X	X	VI	VI
VII	VII	VII	VII	VII	VII	VII	VIII	VII	VII	VII	VII
VIII	VIII	VIII	VIII	VIII	VIII	VIII	VII	VIII	VIII	VIII	VIII

bases on the other, extending over a period of four years. According to the relative fertility of the plots, as determined in the beginning of the experiment, the experimental area has been divided into two sets, (A) comprising I—V plots and (B) VI—X plots. The plots have been arranged in vertical columns according to their values in descending order.

From the scheme it will be seen that the usual arrangement in the treated plots in (A) up to the end of second treatment is I, V, II, but in subsequent treatments

plot V having given higher yields than plot I, the arrangement becomes V, I, II. Plots III and IV, being control and leached plots and there being hardly any difference between their treatments, change place occasionally.

In B, the usual arrangement in the treated plots based on crop yields up to the end of second treatment is IX, X and VI, but later on plot VI, having done better than X, the arrangement becomes IX, VI, X. Based on ratios alone, plots VI and X change places occasionally. The controls and leached plots, as usual, are worse than treated plots and the usual arrangement throughout is VII and VIII.

This scheme clearly shows that on the whole there is a fairly close agreement between the fertility of plots as determined by crop yield and the ratios of divalent to monovalent bases.

Dispersion Coefficient.

Puri and Keen [1925] and Puri [1930] have shown that dispersion coefficient can be used for comparing the state of aggregation of soil particles in different soils. It is a matter of common observation that soil particles in most of the alkali soils are so closely cemented with one another, that they render the soil entirely impermeable to water, but as already shown, when these soils are treated with calcium salts, the physical condition improves considerably and they become more permeable to water (see moisture curves, Fig. 2). Therefore, it seemed feasible to seek for a relationship between the dispersion coefficient of soils under examination and the Ca-Na ratio in the exchange complex as already determined and reproduced again for the sake of comparison in Table IX.

TABLE IX.

Plot No.	Ratio of calcium to sodium.		Dispersion coefficient.		pH value	
	Before 1st treatment	After 4th treatment	Before 1st treatment	After 4th treatment	Before 1st treatment	After 4th treatment
I	19 : 81	46 : 54	52·50	21·62	8·66	8·09
II	22 : 78	43 : 57	43·72	25·62	8·70	8·32
III	20 : 80	17 : 83	49·23	87·95	8·50	9·41
IV	21 : 79	20 : 80	68·60	91·22	8·80	9·47
V	20 : 80	58 : 42	78·38	45·09	8·90	8·60
VI	11 : 89	51 : 49	75·16	31·15	9·30	8·70
VII	11 : 89	12 : 88	70·07	97·58	9·10	9·68
VIII	13 : 87	10 : 90	68·62	90·18	9·10	9·71
IX	11 : 89	89 : 11	79·67	20·61	9·10	8·22
X	10 : 90	72 : 28	83·88	40·28	8·90	9·03

The determinations were made according to Puri's method, but it was observed that like moisture content, the presence of soluble salts, especially those of sodium and calcium caused considerable variations in the dispersion coefficients. Leaching of the soluble salts with water, on the other hand, does not improve matters, as it is apt to disturb the equilibrium existing between the forces of cohesion and dispersion of the soil particles. However, the investigation is still in hand and it is hoped to overcome these difficulties and devise a method for determining soil fertility based on dispersion of soil particles. The results of dispersion coefficient with the present method give at best a comparative value of the relative fertility of the different plots, but do not furnish any definite quantitative data.

Tube Experiments.

In order to determine the exact dose of calcium salts required for maximum displacement of sodium by calcium in a *bari* soil, experiments were conducted in percolation tubes specially prepared for the purpose. Similar experiments were also carried out with a normal soil by treating it with sodium salts in order to study the reversal of the above reaction.

Glass tubes of equal bores and 1 ft. in length were uniformly packed with 200 grms. of *bari* soil, and two tubes of the same dimen sions with equal amount of normal soil. The lower ends of the tubes were fitted up with rubber stoppers through which passed a small glass tubing of equal and uniform bore. Between the rubber stoppers and the soil a packing of glass wool was interposed. This prevented the washing down of soil particles during the process of leaching, and quite clear percolates were obtained with the help of this device.

Bari soil was leached with calcium chloride solution and normal soil with sodium chloride solution. Leaching was continued to a state of saturation in each case, and this was determined by estimating the amount of calcium present in the percolates. Altogether 200 c. cs. of calcium chloride and 200 c. cs. of sodium chloride were required to complete the reaction.

The amount of exchangeable bases together with ratio of divalent to monovalent bases determined in the soils after the completion of the reaction are given in

Table X. The results clearly show that a high ratio of divalent to monovalent bases contributes to the fertility of the soil and *vice versâ*.

TABLE X.

(*Tube Experiments.*)

Exchangeable bases as percentage on air-dried soil.

Serial No.	Description of the soil	Calcium as Ca	Magnesium as Mg	Sodium as Na	Potassium as K	Ratio of divalent to monovalent bases		Ratio of calcium to sodium		Dispersion coefficient
						Divalent	Monovalent	Ca	Na	
1	Calcium chloride treated soil	0·1440	0·0099	0·0029	0·0144	90	10	97	3	28·0
2	*Bari* soil untreated	0·0400	0·0122	0·1599	0·0378	21	79	20	80	93·7
3	Normal soil treated with sodium chloride	0·0200	0·0070	0·1167	0·0268	16	84	15	85	82·0
4	Normal soil untreated	0·1120	0·0144	0·0240	0·0234	73	27	82	18	43·0

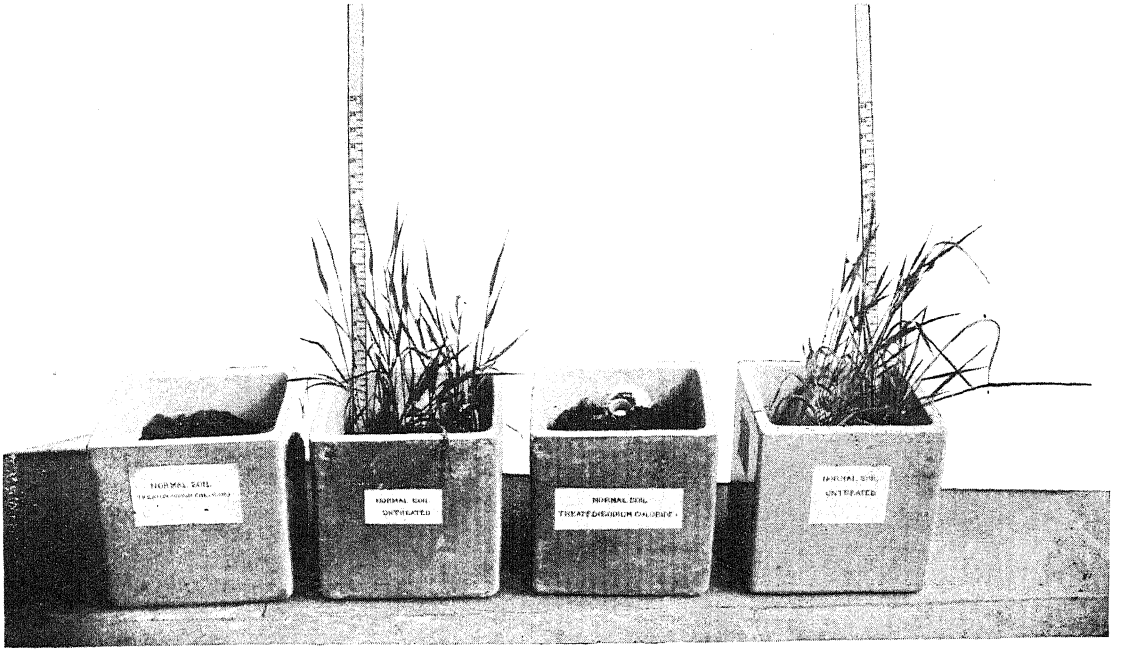
Pot Experiments.

On the basis of the results obtained from the tube experiments 666 grms. of calcium chloride and 1032 grms. of gypsum (containing equivalent amounts of calcium), were added to 12,000 grms. of *bari* soils and placed in earthenware pots (diagram). A similar treatment with 696 grms. of sodium chloride was given in 12,000 grms. of normal soil. The chemicals were left over with the soil in moist condition for about 4 months and during this period the soils were occasionally watered and stirred up in order to ensure thorough mixing up of the chemicals with the soil. Eventually the soils were thoroughly leached and wheat was sown. (Pl. 1., figs. 1, 2). Table XI depicts the ratio of exchangeable bases tabulated against crop yield and dispersion coefficient of the soils. *Bari* soil as such and normal soil treated with sodium chloride do not give any yield at all. A second statement of crop yield for the year 1930-31 shows the possibility of greatly improving a calcium-treated soil with an average dose of farmyard manure. The results clearly show a distinct correlation between the crop yields on the one hand, and the ratio of divalent to monovalent bases and the dispersion coefficient on the other. A high



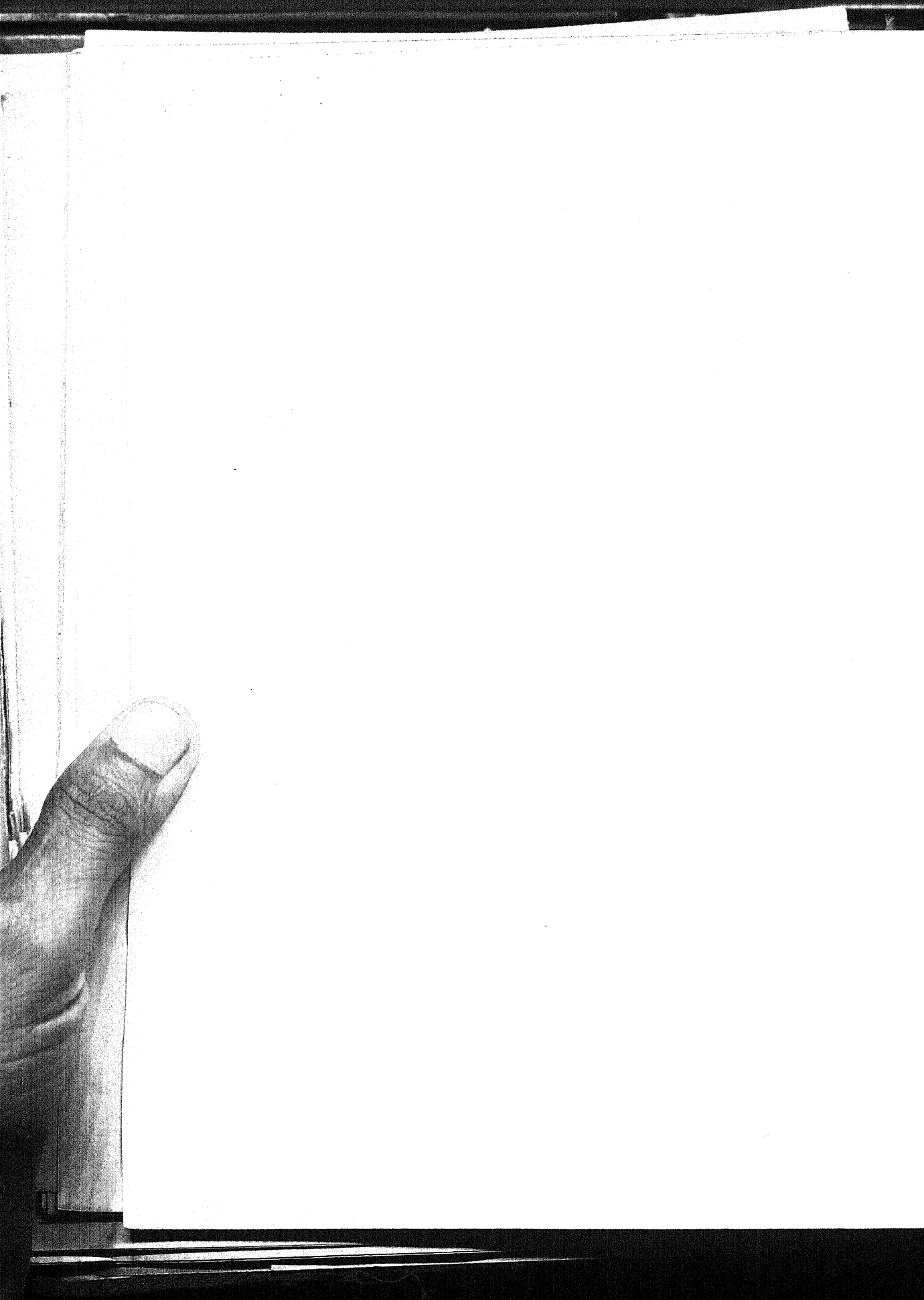
Bari soil untreated.	Bari soil treated (calcium chloride).	Bari soil treated (flooding and washing).	Bari soil treated (gypsum).

Fig. 1.



Normal soil treated (sodium chloride).	Normal soil untreated.	Normal soil treated (sodium chloride).	Normal soil untreated.

Fig. 2.



ratio of divalent to monovalent bases and a low dispersion coefficient gives a clear indication of the fertility of the soil and *vice versâ*.

TABLE XI.

(*Pot Experiments.*)

Ratio of divalent to monovalent bases, of calcium to sodium and dispersion coefficient tabulated against crop yield.

Serial No.	Description of the soil	Ratio of divalent to monovalent bases				Dispersion coefficient	Total yield of crop without farmyard manure	Total yield of crop with farmyard manure
		Divalent Ca, Mg	Monovalent Na, K	Calcium Ca	Sodium Na			
							1929-30.	1930-31.
1	*Bari* soil treated with calcium chloride	82	18	92	8	16·26	10·30 grms,	17·7 grms.
2	*Bari* soil treated with gypsum	55	45	59	41	24·80	6·84 „	14·3 „
3	*Bari* soil control	10	90	8	92	94·68	*nil*	*nil*
4	*Bari* soil flooding and washing	15	85	12	88	82·46	*nil*	*nil*
5	Normal soil treated with sodium chloride	21	79	23	77	96·28	*nil*	*nil*
6	Normal soil treated with sodium chloride	23	77	22	78	96·68	*nil*	*nil*
7	Normal soil control	70	30	84	16	36·53	13·03 grms.	11·2 grms.
8	Normal soil control	66	34	63	37	40·38	10·36 „	9·8 „

Summary.

1. There are different types of *kallar* soils which require different treatments for their reclamation. Therefore a preliminary study regarding the nature and origin of *kallar* is a first essential towards any method of reclamation.

2. In certain soils excess of sodium salts react with the clay fraction, and produce what is known as sodium clay. In order to improve these soils the first essential is to convert sodium clay into calcium clay with the help of calcium salts.

3. Leaching and drainage alone in such soils cannot bring about any permanent cure, and may do more harm than good in the long run.

4. The extent of *kallar* trouble in such soil is measured by a ratio of sodium to calcium or a ratio between the monovalent and divalent bases present in the exchange complex.

5. The amount of calcium salts required for the reclamation of such soils depends on the sodium present in the exchange complex.

6. The dispersion coefficient may furnish as a ready method for determining the relative amounts of exchangeable calcium and sodium in the *kallar* soils.

7. With the help of calcium salts followed by a treatment of farmyard manure it is possible to improve the *bari* soil to the status of a normal soil.

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REFERENCES.

1. Hibbard (1921). *Soil Sci.* **11**, 385—387.
2. Hibbard (1922). *Soil Sci.* **13**, 125—134.
3. Hissink (1923). *Soil Sci.* **15**, 269—276.
4. Joffe and McLean (1924, 1). *Soil Sci.* **17**, 395—409.
5. ——————— (1924, 2). *Soil Sci.* **18**, 13—30.
6. ——————— (1924, 3). *Soil Sci.* **18**, 133—150.
7. ——————— (1924, 4). *Soil Sci.* **18**, 237—254.
8. Kelley (1922). *Soil Sci.* **14**, 177—190.
9. Kelley and Brown (1925). *Soil Sci.* **20**, 477—495.
10. Kelley and Chapman (1930). *Soil Sci.* **30**, 391—406.
11. Lipman (1916). *Soil Sci.* **2**, 205—207.
12. Puri and Keen (1925). *Jour. Agric. Sci.* **15**, 147—161.
13. Puri (1930). *Memoirs of Dept. Agric. India* **11**, 39—51.
14. Snyder (1928). *Soil Sci.* **26**, 107—111.

SUGARCANE-SORGHUM HYBRIDS.

PART I. GENERAL OUTLINE AND EARLY CHARACTERS.

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(With Plates II to VIII.)

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I. INTRODUCTION.

The sugarcane is a long-duration crop all the world over, occupying the land for as much as twenty-four months in Hawaii. In India the cane is on the land only from twelve to fourteen months; but, even so, the period often corresponds to that of two other short duration crops. In the past, attempts have been made both in India and elsewhere, to evolve short duration canes chiefly for extending the period of crushing in the factories.

Short duration canes have a special use in sub-tropical regions like Louisiana and North India on account of the rather limited available growth period for canes

in such regions. In parts of North India, for instance, the cane is planted in the ground even as late as the middle of March. High temperatures without humidity —unfavourable conditions for cane growth—prevail in these parts till about June. The monsoon months of June to September, with their high temperatures combined with high humidity, are the months most favourable to cane growth; but these are soon followed by the winter months of low temperatures which are again unfavourable for such growth. It will thus be seen that canes in such regions barely get a six month period in which to build up tonnage and ripen the juice.

To try and meet this situation, the Sugarcane Station at Coimbatore—which had been charged with the task of breeding improved canes for every part of India —had kept in view the evolving of early maturing or short duration varieties; and a certain amount of success has been attained. The Coimbatore canes—Co. 214, Co. 281 and Co. 290—are examples of such breeding. These have been welcomed in sub-tropical North India as, with these canes, it has been possible to start the factories or the manufacture of *gur* (unrefined sugar) by the indigenous methods earlier than with the other extant kinds.

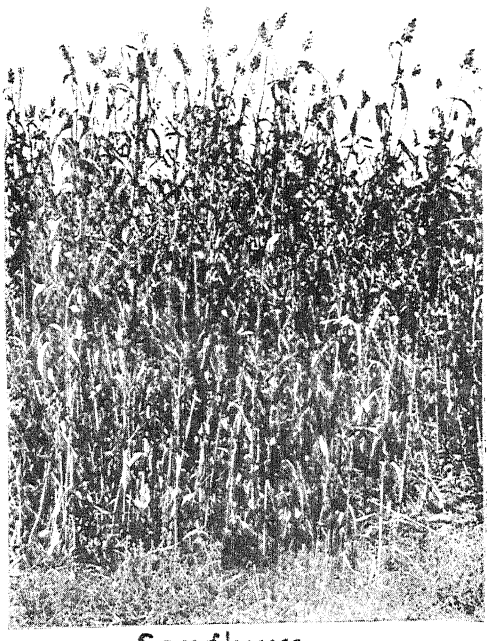
II. Object of the hybridization.

For some little time past, it has been the ambition of the authors, however, to breed canes which planted in June would mature in November or December in North India. It was argued that such canes would save the grower in North India the care and attention that are needed by the present cane crops during the difficult summer months from March to June. A careful examination of the available sugarcane parents showed that none of them was likely to be useful for the purpose as even the earliest of them needed about ten months to ripen. This led the authors to turn their attention to the available cereals, which complete their life cycles in less than six months. It was hoped that such hybridization, if successful, might yield short duration sugarcanes maturing in about six months. The first attempts at these intergeneric hybrids with the sugarcanes included not only cereals like *jowar* (*Andropogon Sorghum*) and *cumbu* (*Pennisetum typhoideum*) but also certain weeds like *Cyperus retundus* and *Pennisetum Alopecuros*. Undoubted hybrids were however obtained only with *jowar*.

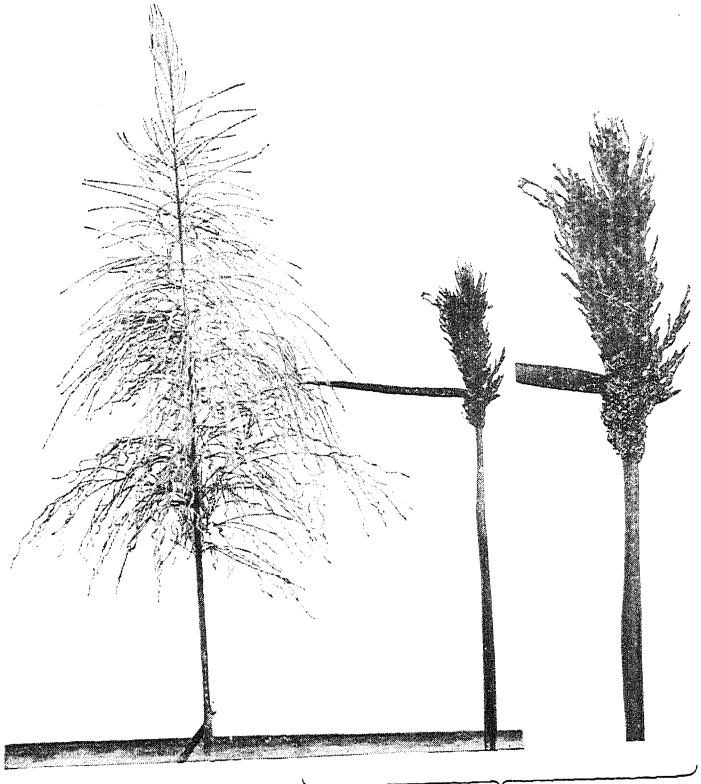
During the first attempts the writers were not quite hopeful of success. Though reports on intergeneric hybrids have been current for almost a century, authenticated cases have been rare [Babcock and Clauson, 1927]. The orchid family has been pre-eminent in this respect yielding a large number of ornamental types. Perhaps the two most outstanding instances of intergeneric hybridization connected with crop plants are the maize-teosinte hybrids [Collins and Kempton, 1920] and hybrids between wheat and rye reported by Wilson as



Sugarcane

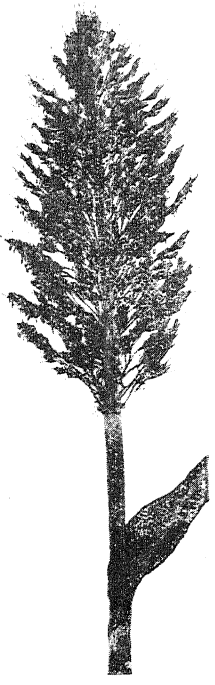


Sorghum

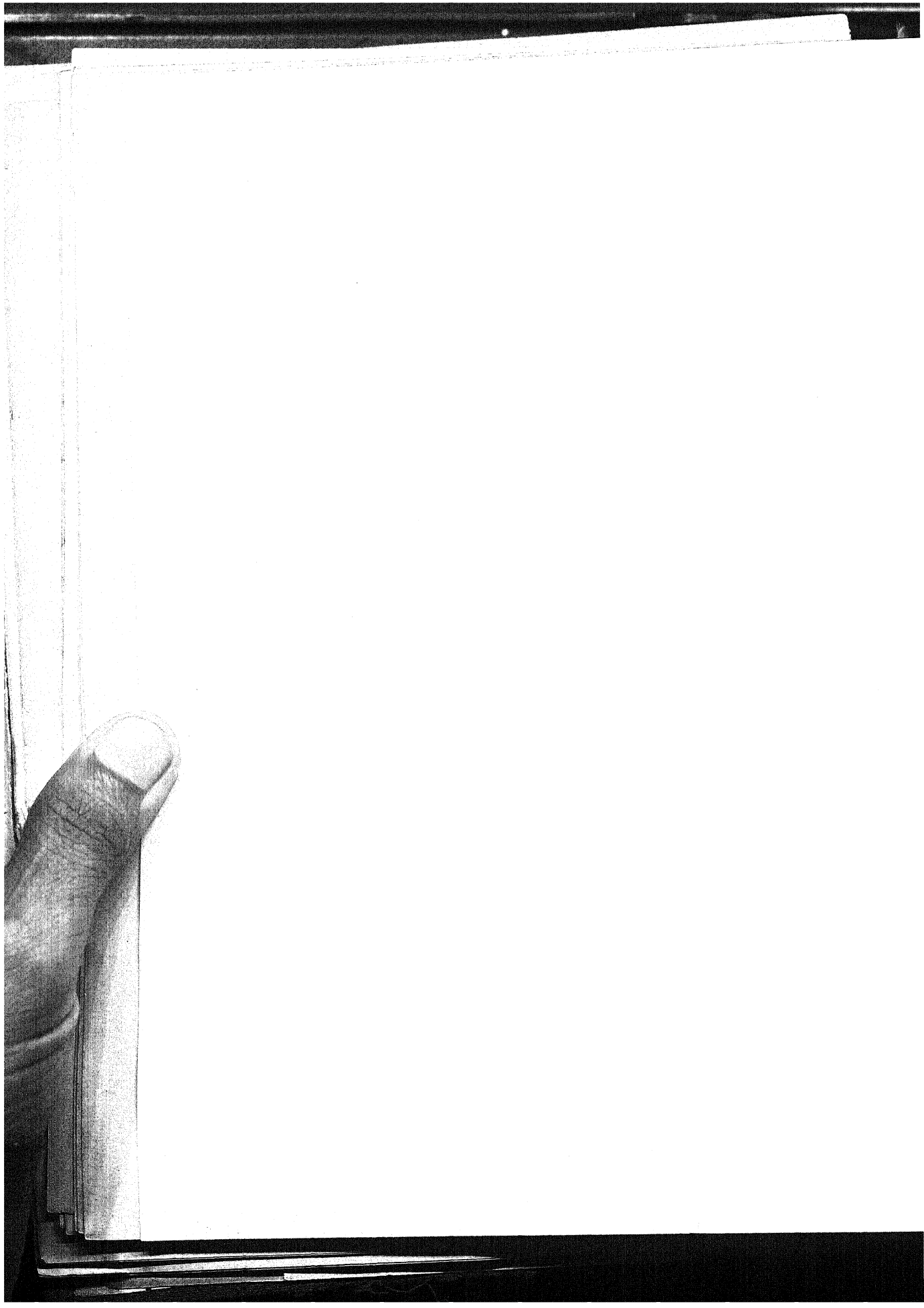


Arrow of P. O. J. 2725.

P. O. J. 2725 × Sorghum Hybrid No. 50.



Sorghum.



early as 1875. Considerable work has developed on the latter of these hybrids [Love and Craig, 1919 ; Gaines and Stevenson, 1922]. Within recent years interesting work on the nuclear divisions in the pollen mother cells of intergeneric hybrids between *Triticum, Aegilops* and *Secale* has been published [Longley and Sando, 1930]. The work reported in the present paper differs somewhat from those above mentioned in the fact that at least one of the parents employed, the sugarcane, is capable of propagation by the vegetative method. Many of the hybrids obtained have shown a similar capacity. The sterility of the flowers, frequently associated with such hybrids, is not in this case as much an obstacle for the securing of economic results as in the case of crops propagated from seed.

III. Selection of parents.

P. O. J. 2725 was selected as the mother parent because (1) it is a desirable type of cane and (2) this variety, besides being practically devoid of healthy pollen of its own, has been known to seed freely at Coimbatore on hybridization. The male parent employed was the sorghum known as '*periamanjal*' round about Coimbatore, the pollen of which was easily available at the time. For brevity this will be referred to merely as '*periamanjal*' in these papers. Mr. G. N. Rangaswami Ayyangar, Millet Specialist at Coimbatore, has kindly informed me that the correct botanical name of this sorghum as given by Kew is *Sorghum Durra* Stapf.

IV. Breeding technique employed.

As both emasculation and bagging have been giving but poor results at Coimbatore [Venkatraman, 1925] they have been discarded for hybridization work some time back. The first trial cross-pollinations between sugarcane and sorghum were done by isolating the mother arrows, well before their emergence from the enclosing leaf sheaths, in places far removed from access of unintended sugarcane pollen and dusting the stigmas when ready with the desired pollen. [Venkatraman and Thomas, 1926.] Other arrows of the mother similarly isolated but left untreated with any pollen were used as controls. In the present case, whereas the controls on sowing gave no germinations, those hybridized with sorghum pollen gave free germination in the pans, sometimes a hundred seedlings per pan. The pans used were shallow, circular, earthen pans about five inches high and thirteen inches in diameter.

After assuring ourselves that the *periamanjal* does hybridize with P. O. J. 2725 and form fertile seeds, the following technique was employed to raise these hybrids on a bulk scale. Sorghum earheads, with the anthers in condition to open the next morning, are collected the previous evening, leaves and adhering sheaths suitably trimmed for convenience in handling and the earheads kept in a cool shed

with the bases of the stalks under water in earthen pots (Pl. III, fig. 1). Early next morning at about six, these earheads are taken out of the earthen pots and tied into convenient sized bundles. These bundles are then wrapped round with tissue paper and placed for a time on wooden benches under shade. By about 7 a.m. the tissue paper packets are found to contain plenty of pollen, most of it sticking to the inside of the opened anthers and a small amount adhering to the paper wrappers on the inside (Pl. III, fig. 2). When the cane stigmas are ready to receive pollen, the bundles of sorghum earheads are taken to the mother arrows, the paper wrappers discarded, and the earheads gently tapped in the vicinity of the arrows intended to be crossed. A cloud of pollen is now seen to leave the anthers of sorghum and settle on the cane stigmas; and in about two to three hours the sorghum pollen could be seen germinating on the cane stigmas. The above technique, though perhaps simple, needs some experience and delicacy of handling for satisfactory results.

V. Juice analysis of the six-month canes.

The resultant hybrids—even the very first lot—have shown considerable promise in realizing the object with which the crossing was effected. Over a dozen of the hybrids matured in five or six months—both as seedlings and as sett plants—and yielded satisfactory juices at the end of that period (Table I).

Table I.

Juice analysis of sugarcane-sorghum hybrids, six months old.

Seedling No.	Brix	Sucrose	Glucose	Coefficient of purity
	per cent.	per cent.	per cent.	
Co. 351	20·42	18·53	0·17	90·8
Co. 352	19·31	17·33	0·26	89·6
Co. 353	19·01	16·75	0·42	88·1
Co. 354	18·38	16·18	0·41	88·0
Co. 355	17·71	15·22	0·58	85·9
Co. 356	18·51	16·11	0·50	87·0
Co. 357	20·15	18·00	0·18	89·3

The seedlings in the above table possess satisfactory agricultural characters as well. Still better juices were obtained from certain others of these hybrids,



Fig. 1. Young sorghum earheads being trimmed and preserved for use in pollination next morning.

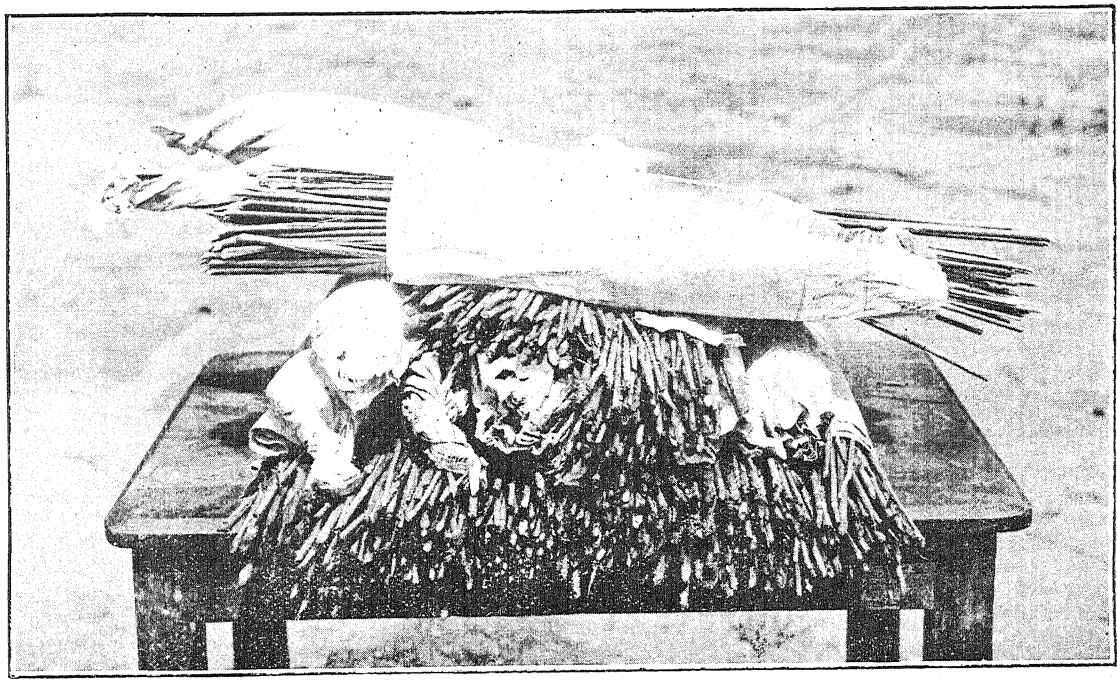


Fig. 2. Bundles of sorghum earheads ready to be used for pollination

PLATE IV.

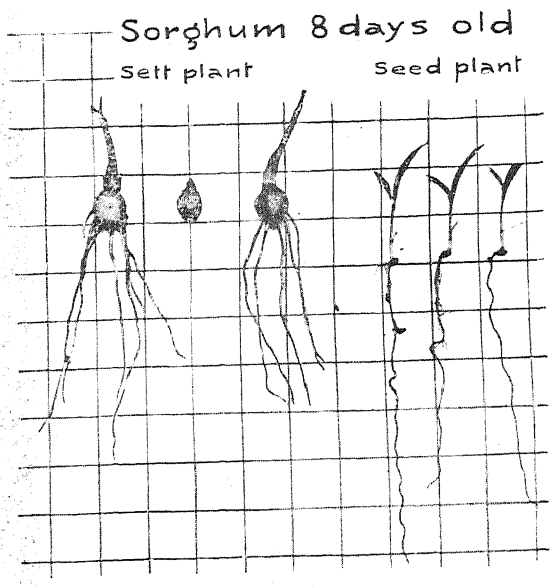


Fig. 1.

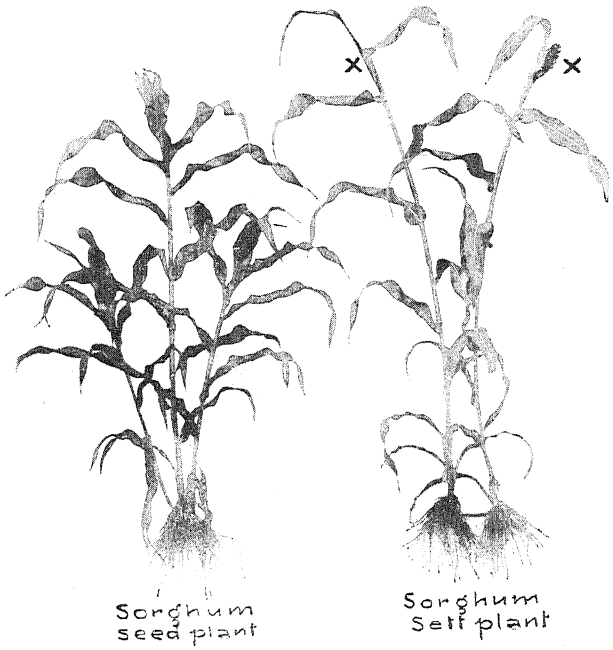


Fig. 2.
Sorghums from cuttings flowered a fortnight earlier than seed grown sorghums.

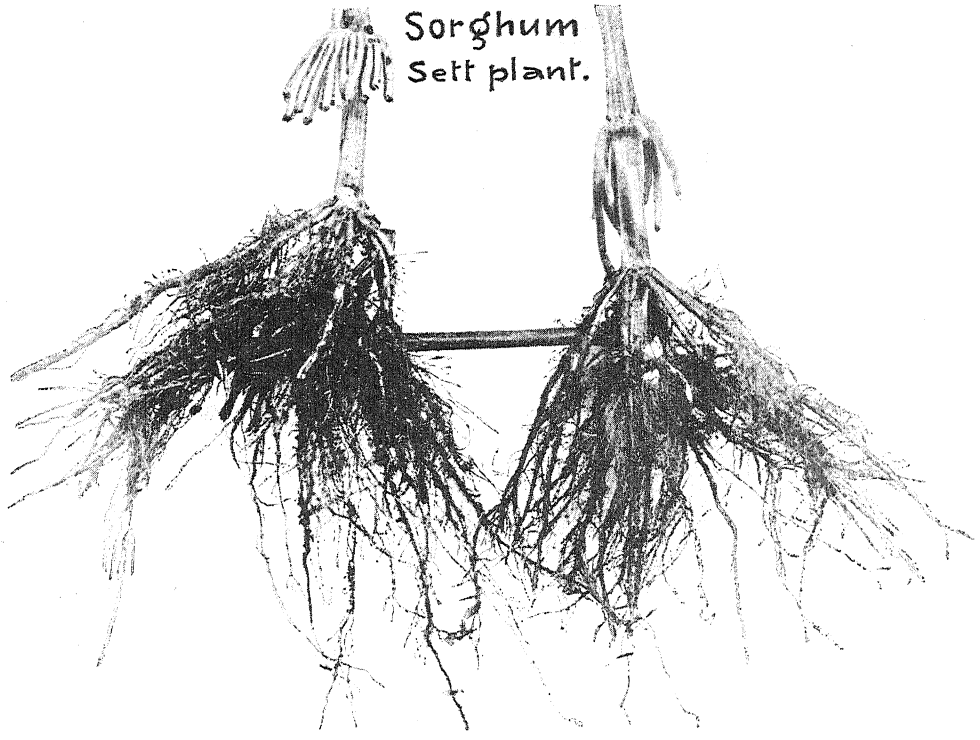


Fig. 3.
Sorghum grown from a cutting and showing the characteristic " Stilt " roots.

but these were unsatisfactory from an agricultural viewpoint, chiefly low tonnage. The above analyses are of fair sized samples, all the canes in a twenty-foot row of each being cut for crushing.

VI. Vegetative propagation of the hybrids and of sorghum.

It was early realized that, to be useful in cultivation, the hybrids should be capable of propagation in the vegetative way from setts or cuttings, as this would ensure in practically every individual of the field population all the good characters of the new types. From the presence of root zone and bud at every node and other similarities with the sugarcane, there appeared to exist little need for doubt on this score. Soon after cane formation, immature canes from half a dozen of the hybrids were cut into setts and planted. These soon grew up and tillered like the sugarcane, placing beyond doubt the possibility of vegetative propagation of these hybrids.

This led to an examination of the sorghum stem which showed a general resemblance to the cane ; and it was found that, with properly selected material, the sorghum also could be grown from cuttings. Thus grown the sorghum plants showed slightly broader leaves in the earlier stages. In one series of plots the crop from cuttings flowered about a fortnight earlier than that from seed (Plate IV, fig. 2). Mr. G. N. Rangaswami Ayyangar informed me that the multiplication of sorghum from cuttings was done successfully by Andre Piedallu, a Frenchman, in 1919. It has been doubted in competent quarters if the growing of sorghum from cuttings will have any use in field practice. One possible use would be in cases where it is desired to multiply a particular sorghum plant, without any risk of losing through seed the special peculiarities of the plant.

VII. General appearance of the hybrids.

In looking over the hybrids from time to time the question naturally suggested itself "Do these intergeneric hybrids take more after the sugarcane or the sorghum ?". At the outset it has to be mentioned that none of the hybrids grew as rapidly as the sorghum, though a certain number of them did finish their life-cycles much earlier than the sugarcane parent or any of the cane hybrids with that parent. The sorghum parent is practically devoid of tillers but most of the hybrids started tillering like the cane, some of them at a very early stage. Though the hybrids included types never before met with in sugarcane seedlings, yet there were quite a number of plants which grew like the cane and analysed like it, certain of these combining the early maturity for which the crossing was originally effected. When it is remembered that the hybridization was made for breeding sugarcanes and not *jowars*, it will be seen that the results have been in the direction desired.

VIII. Brief résumé of evidence about the genuineness of the hybrids.

As to the plants being genuine hybrids between sugarcane and sorghum there was little room for doubt after the plants had grown a little. Traces of sorghum blood were clearly seen in the vegetative and crop characters of the hybrids some of which are mentioned below.

1. Shape, texture and surface of the leaf.
2. Behaviour of leaf on fading.
3. Single row of root eyes on the root zone.
4. Shape, size and mode of development of buds.
5. Short duration of the crop never before met with in sugarcanes.

Such evidence increased considerably when the hybrids began to flower. A few of them carried sorghum-like earheads at the tops of sugarcane stems. Sorghum blood was further traceable in definite morphological characters like (1) an awned fourth glume, (2) solid nature of the main axis, (3) shape of lodicules, and (4) presence of neuter flowers. The hybrid nature of the plants was further confirmed by cytological studies of the chromosomes. All such data with illustrations will be presented in this and subsequent papers.

IX. Early characters.

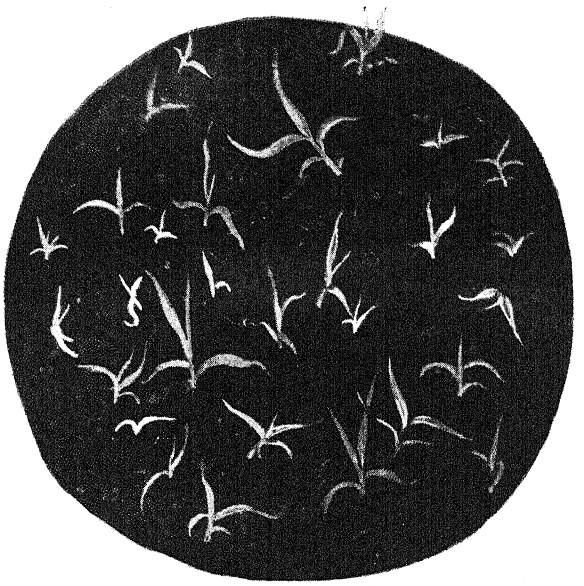
To enable constant comparison the sorghum hybrids were planted in the field between two sugarcane hybrids of P. O. J. 2725, *viz.*, P. O. J. 2725×B. 3412 and P. O. J. 2725×66 White Carp, of about the same age. Besides the above, hybrids between P. O. J. 2725 as mother and six other sugarcanes—Badila, Q. 813, Co. 214, Co. 213, Co. 281 and Co. 290—were available for comparison nearby, these crosses having been effected by Mr. N. L. Dutt, the Second Cane Breeding Officer, in the course of his breeding work. There was thus plenty of suitable material for instituting valid comparisons at every stage during the growth of the hybrids.

(*a*) *Shape of first leaf.*

Soon after germination, the broader and shorter first leaves of the sorghum hybrids marked them off from the narrower and longer leaves of the cane hybrids. The leaves of the sorghum hybrids were further noticed to be lighter green and softer to the feel. The first leaves of sorghum, whether grown from seeds or cuttings, are lighter green and softer to the feel than those of germinating sugarcanes. It was found rather difficult to illustrate these differences, though clearly and easily seen in the field ; but an attempt has been made to do so (Plate V).

(*b*) *Occurrence of albinos.*

In three to four weeks the sorghum hybrids showed quite a large number of albinos—eight per cent. in one batch—while the cane hybrids with P. O. J. 2725

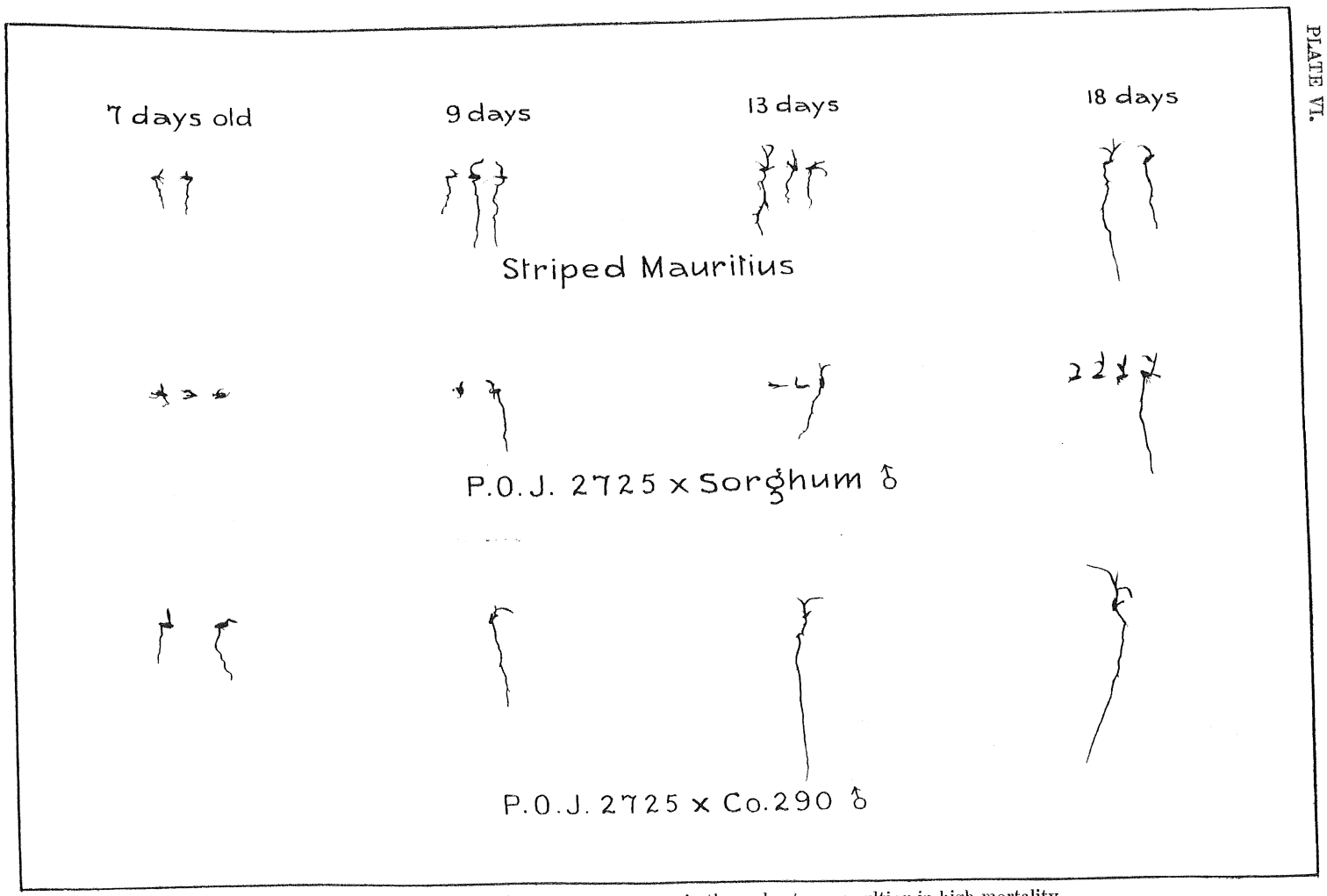


P. O. J. 2725 × Sorghum (Periamanjal)
30 days old.



P. O. J. 2725 × B. 3412
30 days old.

In the early stages the sorghum hybrids are broader leaved, lighter green in colour, softer to the feel and slower in growth. They also contain "albinos" not met with in sugarcane hybrids with P. O. J. 2725.



The sorghum hybrids exhibit a poor root-system in the early stages resulting in high mortality.

did not show any (Plate V). Albinos has been reported as common in certain seed-grown sorghums. [Conner and Karper, 1924.] In a kind communication to the senior author, Dr. E. W. Brandes of the Department of Agriculture, U. S. A., states that albinos have been noticed in sugarcane seedlings at Porto Rico by Mr. H. P. Cowgill. During their twenty years experience of sugarcane breeding at Coimbatore the authors have not noticed albinos in cane seedlings. The difference between the experiences of Porto Rico and Coimbatore is perhaps due to difference in the kind of parents employed. In spite of considerable care and attention, it was not possible to save any of the hybrid albinos. Certain of the hybrids with pale green leaves did recover after a time, but showed appreciable lack of vegetative vigour throughout their growth.

(c) *Colonization by* Aphis maidis.

During the younger stages of growth of these hybrids, when doubts were entertained by the authors about the genuineness of the hybrids, the insect *Aphis maidis* gave an indication of some significance. For constant comparison, both the parents, as also two of the cane hybrids with P. O. J. 2725, were planted on land contiguous with the hybrids.

After a couple of months from germination a fair amount of *Aphis maidis*—kindly identified as such by Rao Sahib Y. Ramachandra Rao, Government Entomologist at Coimbatore—was found to have colonized on the sorghum plants and on a few of the sorghum hybrids (half a dozen out of two hundred). The plants of P. O. J. 2725, as also the cane hybrids with P. O. J. 2725 (about three hundred in number), were found completely free. Here is perhaps an illustration of an insect being able to discover the real nature of a population quicker than the morphological botanist.

(d) *High mortality among hybrids traced to a poor early root-system.*

From about the third week the hybrids began to die off in large numbers. As illustrative of the high mortality it might be mentioned that during the arrowing season of 1930, whereas 70,000 germinations were secured, it was found possible to plant in the second nursery only about 4,000. This mortality was not confined to the albinos alone, but extended even to seedlings possessing normal green leaves. Careful root dissections in the field showed that this mortality was due to the very poor development of the early roots in most of these hybrids (Plate VI). An early and strong root-system is a very desirable character in sugarcanes as it contributes to a good and healthy stand in the field. If all the F_1 plants of this hybrid were to show such a character of the roots, the sorghum hybridization would need to be given up as unsuited for the breeding of economic types. Fortunately, however,

the intergeneric hybrids showed considerable variations in this respect; and certain of the surviving ones exhibited quite an early and good root-system.

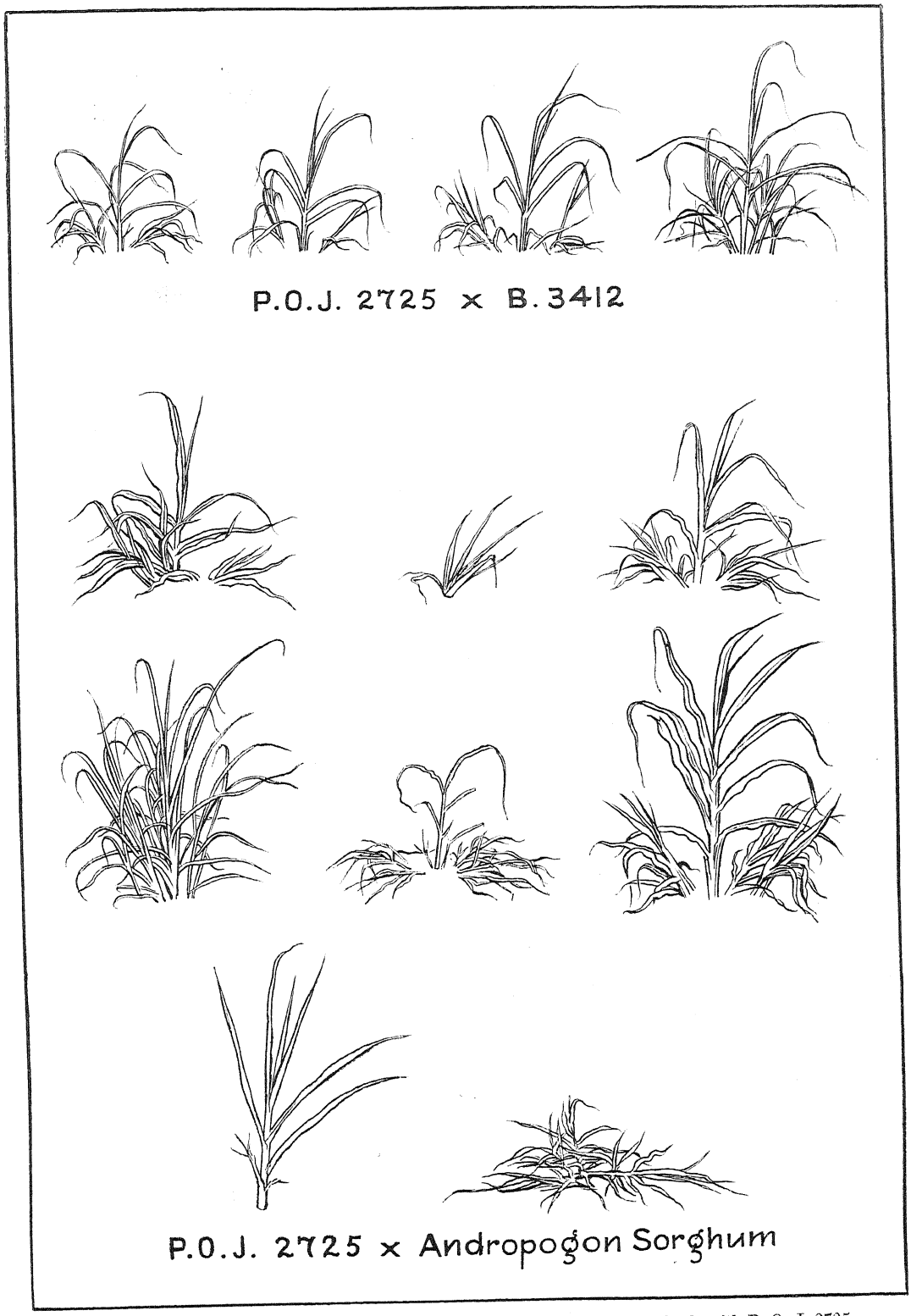
(e) Wide variations in the hybrids.

When sugarcane is grown from seed instead of from cuttings—the usual method of propagation in the cultivation of this crop—considerable variations are noticed in the seedlings even when adequate precautions are taken for selfing the mother arrows. This results in practically as many types as the seedlings raised. The success of cane-breeding is, in fact, directly dependent on the frequency and range of such variations [Venkatraman, 1930]. This places the breeding of sugarcanes in a class by itself and the familiar methods of handling the material or presenting results now associated with the breeding of most other crops are inapplicable to the cane [Venkatraman, 1927]. When two cane varieties are crossed with each other, this variation is generally enhanced resulting in a wider range of types. The sugarcane-sorghum hybrids showed still greater variations, and many curious forms were noticed in the hybrid population (Plates VII & VIII). One seedling out of a thousand in one batch was very narrow-leaved and looked like a weed grass. The hybrids included many lop-sided forms and quite a large number developed into obviously uneconomic types with broad thickish leaves, little cane formation and spreading close to the ground (Plate VIII).

(f) Water requirements of the hybrids.

During the twenty years of its existence the Coimbatore Station had developed a satisfactory technique for growing young sugarcane seedlings to full maturity. All the seedlings bred at the Station are treated in the same manner and no difficulties were experienced so far. This technique proved however unsuitable to these sorghum hybrids; and it was not till a change was effected that the hybrids grew normally.

For some time after planting in the ground the cane seedlings used to be irrigated, once in three to four days, according to prevailing weather conditions, the irrigation water being allowed to run on both sides of the plants, which were planted on a ridge in the middle of the furrow. Thus irrigated, the sorghum hybrids showed some yellowing and lack of vegetative vigour suggesting over-watering. This raised the doubt if, in the matter of watering, it was right to treat the new hybrids like sugarcane seedlings. The sorghum parent used needs much less water than sugarcane and is even grown as a rain-fed crop. It was argued that, if any of the hybrids took after sorghum in the matter of water needs, the irrigation by the standard method would be excessive.



Sorghum hybrids show a greater variation of types than the sugarcane hybrids with P. O. J. 2725.

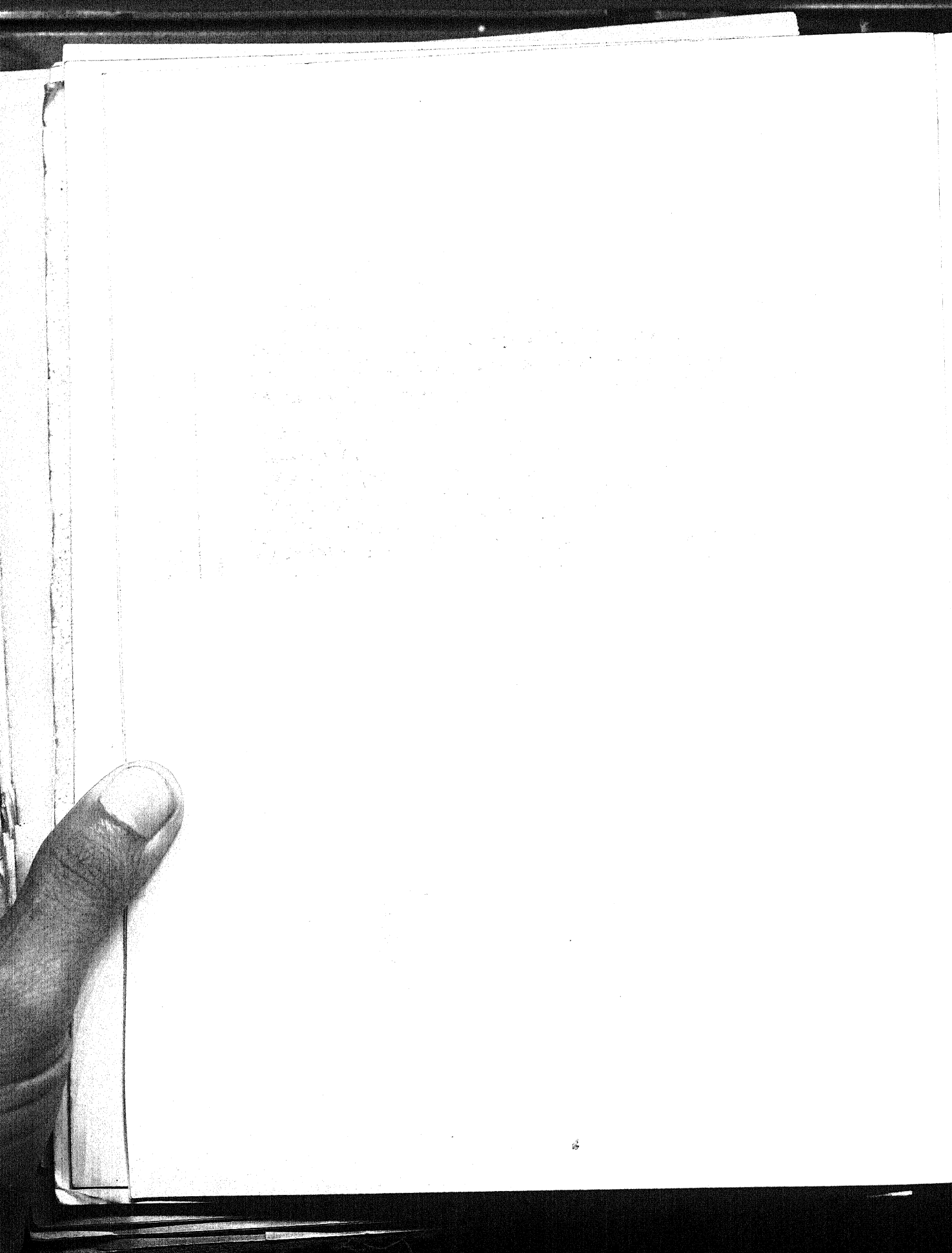




Fig. 1. Many of the sorghum hybrids showed poor vegetative vigour. In this plot of 400 there were only six useful types, one of which is seen in the picture.

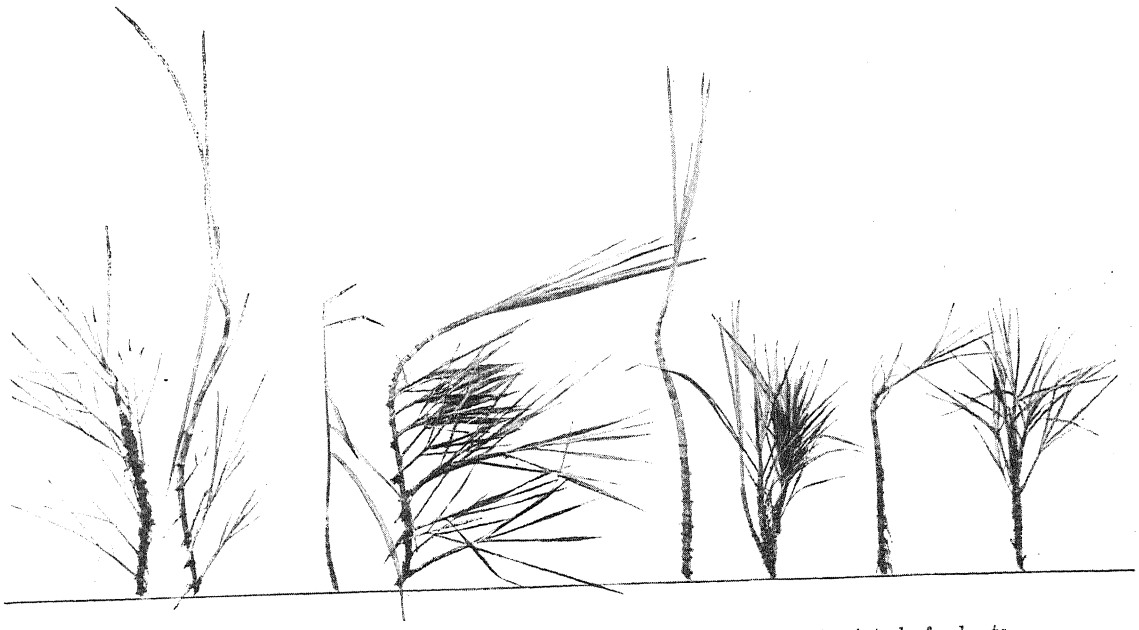
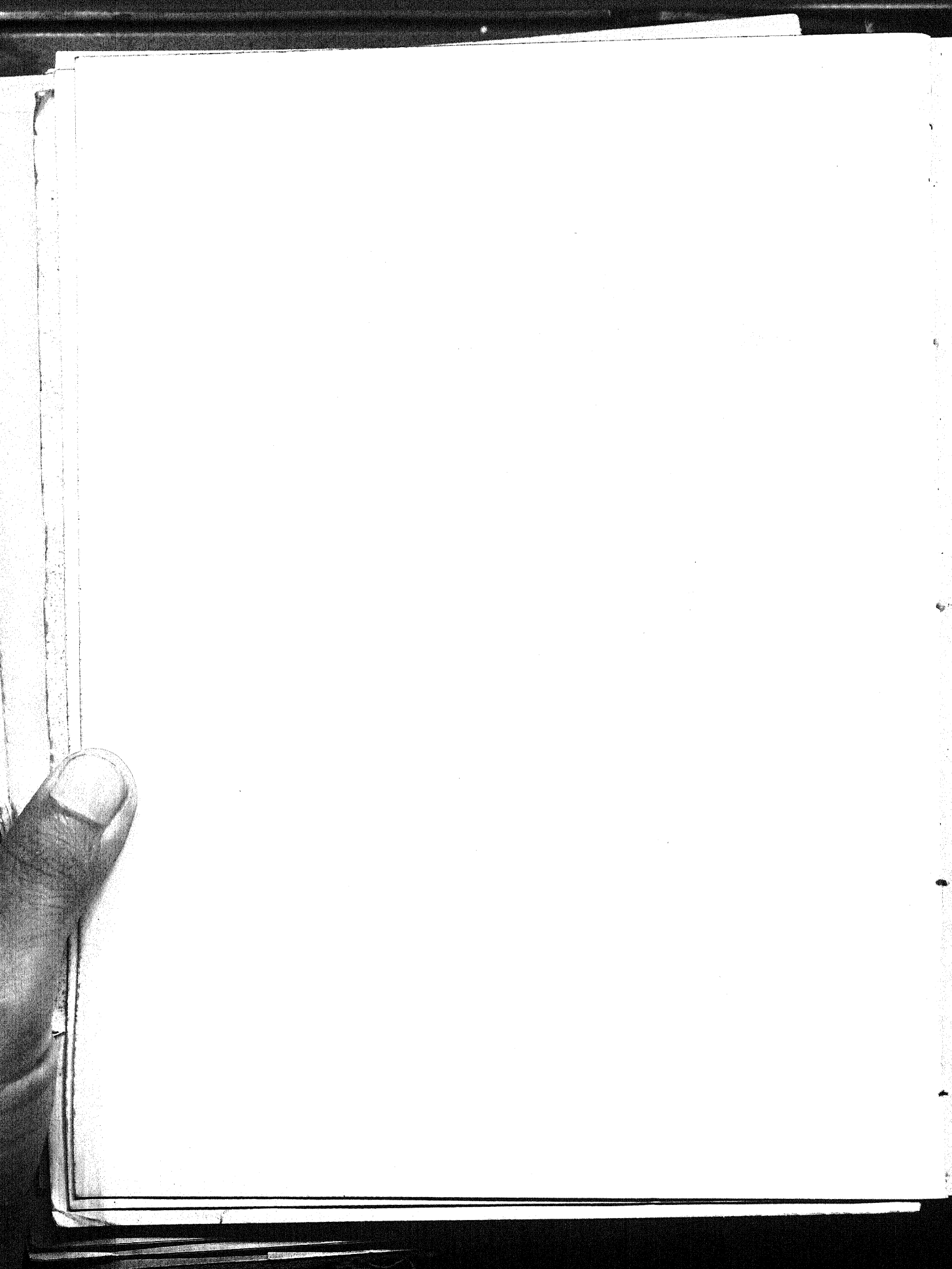


Fig. 2. Certain of the uneconomic cane hybrids. The buds quickly develop into leafy shoots.



A change was introduced by forming the irrigation channel only on one side of the row of plants and working up the other side into a broad raised bed. The irrigation was thus confined to only one side of the plant. It was hoped that such of the hybrids as took after sorghum in the matter of water requirements would develop their roots on the bed or comparatively 'dry' side and those taking after sugarcane on the irrigation or channel side. The arrangement proved satisfactory and the plants began to grow in a normal manner. This has now been adopted as the standard method in preparing plots for sugarcane-sorghum hybrids.

REFERENCES.

Babcock and Clausen (1927). Genetics in relation to Agriculture.
Collins and Kempton (1920). *Jour. Agric. Res.* **19,** 1.
Conner and Karper (1924). *Jour. Heredity* **15,** 377.
Gaines and Stevenson (1922). *Jour. Heredity* **13,** 81.
Longley and Sando (1930). *Jour. Agric. Res.* **40,** 683.
Love and Craig (1919). *Jour. Heredity.* **10,** 195.
Venkatraman (1925). *Agric. Jour. India.* **20,** 173.
Venkatraman and Thomas (1926). *Agric. Jour. India.* **21,** 203.
Venkatraman (1927). *Mem. Dept. Agric. India, Bot. Series* **14,** No. 3.
Venkatraman (1930). *Madras Agric. Jour.* **18,** 417.
Wilson (1875). *Trans. and Proceed. Bot. Soc. Edinburgh* **12,** 2.

A STATISTICAL NOTE ON THE METHOD OF COMPARING MEAN VALUES BASED ON SMALL SAMPLES

BY

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1. Mr. K. V. Joshi, Cotton Physiologist, of the Cotton Research Laboratories, Surat, Gujrat, recently sent me certain data (given in Table I) for the "percentage success of bolls from flowers opening on a plant" for 20 plants each of 5 types of cotton*: (1) A, (2) B, (3) C, (4) D, (5) E, and enquired how the data could be used for comparative purposes.

TABLE I.

Percentage success of bolls from flowers (Surat Cotton) (Mr. K. V. Joshi's Data).

No	A	B	C	D	E
1	43·4	51·8	49·3	39·7	41·5
2	60·0	44·6	63·2	42·0	37·4
3	34·6	43·6	39·2	43·7	42·9
4	33·3	51·3	43·6	52·6	39·8
5	37·5	58·1	47·6	40·9	50·9
6	29·4	55·9	42·9	40·3	49·1
7	35·0	47·2	41·7	48·3	39·3
8	30·6	48·5	55·8	37·5	44·7
9	38·8	43·7	41·9	35·3	37·7
10	38·1	44·6	25·0	35·4	40·3
11	33·7	43·2	55·3	48·0	46·0
12	35·4	47·3	44·6	36·0	52·2
13	33·3	55·6	41·3	37·8	51·0

* As Mr. Joshi's experiments have not yet been concluded I refrain from giving the actual names of the various types of cotton used by him, and have referred to them by the fictitious names A, B, C, D, E. The actual names will become available when Mr. Joshi publishes full details of his experiments.

TABLE I—*contd.*

Percentage success of bolls from flowers (Surat Cotton) (Mr. K. V. Joshi's Data)—contd.

No.	A	B	C	D	E
14	33·8	44·4	52·9	34·9	43·0
15	36·6	41·9	45·5	32·3	36·4
16	33·0	45·5	38·2	35·9	44·4
17	29·8	50·0	51·8	38·7	50·0
18	30·0	44·4	53·0	36·5	50·0
19	35·9	47·9	35·0	40·0	36·1
20	27·3	44·2	51·1	29·2	49·2

2. For the comparison of mean values based on moderately large samples (of size greater than say 25 or 30), it is usually convenient to apply the classical theory of errors *. Let s_1 and s_2 be the standard deviations of the two samples of size N_1 and N_2 respectively. The standard deviations of the respective mean values m_1 and m_2 are then given by $s_1/\sqrt{N_1}$ and $s_2/\sqrt{N_2}$ respectively. The standard deviation of the differences between the two means, that is of $(m_1 - m_2)$ will be given by

$$S_d = \sqrt{\frac{s_1^2}{N_1} + \frac{s_2^2}{N_2}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

in order to test the significance of the differences between the two means we divide $(m_1 - m_2)$ by S_d, and write

$$x = (m_1 - m_2)/s_d \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

Using a table of the probability integral (for example, Table II of the Tables for Statisticians and Biometricians edited by Karl Pearson), it is possible to calculate the probability of occurrences of a difference as large as or greater than the observed difference. (A numerical illustration is given in paragraph 9.)

The observed difference is sometimes taken to be significant when it is twice its standard deviation (*i.e.*, x=2). This corresponds approximately to a probability of 1 in 20, and represents a lower limit below which it will almost never be safe to assert significance. On the other hand observed differences of 2·5 or 3 times the standard deviation may be safely considered significant.

*See discussion in paragraph 12.

3. The classical theory of errors involves three assumptions, namely (*a*) that the errors or deviations from mean values for both the variates are 'normally' distributed (*i.e.*, conform to the Gauss-Laplacian distribution), (*b*) that the two variates under comparison are statistically independent, and (*c*) the observed mean values and observed standard deviations are based on large samples.

It is clear, therefore, that the classical theory of errors cannot be legitimately used for the comparison of mean values based on small samples. During the last 15 years a good deal of theoretical work has been done on small samples by a large number of statisticians. To R. A. Fisher belongs the credit of giving certain general formulæ and also calculating necessary tables for facilitating the comparison of mean values based on small samples. An excellent description of the method is given in Chapter V of Fisher's book: "Statistical Methods for Research Workers"†. This method, however, does not appear to be familiar to all workers in India. A detailed explanation of the numerical calculations may, therefore, prove useful.

4. Let us consider the data given in Table I. We must calculate (*a*) the mean values, and (*b*) the standard deviations for the different strains. For small samples it is usually more convenient to proceed directly (*i.e.*, without grouping). The individual figures in Table I are squared (with the help of a table of squares like Barlow's Tables of Squares, Cubes, etc.), and added. The work is shown in detail for (A) in Table II (*a*). The gross sum of the squares is 26069·51. We require, however, the sum of squares of deviations from the mean. In order to find this quantity we calculate a 'correction' which must be subtracted from the gross sum. The sum of (x) is + 709·5. Squaring 709·5 we have 503390·25. Dividing this quantity by 20 (the size of the sample), we get the correction —25169·51. Applying this correction (*i.e.*, subtracting 25169·51 from the gross

† Fisher's theory is free from the assumption of large samples, but still involves the two other assumptions of (*a*) normal distributions, and (*b*) statistical independence. During the last 5 years the first assumption (of normal populations) has been examined to some extent, and it has been shown that small samples drawn from skew populations (within wide limits) conform to Fisher's theory with closer approximation than to the classical theory. Hence the application of Fisher's theory to small samples from even fairly skew populations would not usually lead to seriously erroneous results. Alternative and more satisfactory tests to suit special purposes have also been devised by Egon Pearson, Neyman and others. But Fisher's test continues to be the most convenient one for every day use. The question of correlation between the variates has just begun to engage the attention of statisticians, and it is not unlikely that further modifications will become necessary when this point is more fully investigated. In fact what is urgently required is a fuller development of the theory of small samples drawn from skew correlated populations.

sum of squares) we obtain finally 900·00 as the sum of squares of deviations from the mean. Also, the mean value is obtained by dividing 709·5 by 20, or 35·475.

TABLE II.

Calculations for Type (A).

No.	(a)		(b)			
	x	x^2	No.	x(+)	x(—)	x^2
1	43·4	1883·56	1	+8·4	..	70·56
2	60·0	3600·00	2	+25·0	..	625·00
3	34·6	1197·16	3	..	—0·4	0·16
4	33·3	1108·89	4	..	—1·7	2·89
5	37·5	1406·25	5	+2·5	..	6·25
6	29·4	864·36	6	..	—5·6	31·36
7	35·0	1225·00	7	0·0	..	0·0
8	30·6	936·36	8	..	—4·4	19·36
9	38·8	1505·44	9	+3·8	..	14·44
10	38·1	1451·61	10	+3·1	..	9·61
11	33·7	1135·69	11	..	—1·3	1·69
12	35·4	1253·16	12	+0·4	..	0·16
13	33·3	1108·89	13	..	—1·7	2·89
14	33·8	1142·44	14	..	—1·2	1·44
15	36·6	1339·56	15	+1·6	..	2·56
16	33·0	1089·00	16	..	—2·0	4·00
17	29·8	884·04	17	..	—5·2	27·04
18	30·0	900·00	18	..	—5·0	25·00
19	35·9	1288·81	19	+0·9	..	0·81
20	27·3	745·29	20	..	—7·7	59·29
	709·5	26069·51	Sum	+45·7	—36·2	904·51
Total .			Total .	+9·5	..	
		—25169·51				—4·51
		900·00				900·00

A great deal of unnecessary arithmetical labour can, however, be usually saved by a slight modification of the procedure.* We first of all subtract a suitable constant quantity, say 35, from all individual figures. The resulting figures (some *plus*, and some *minus*) are given in columns 2 and 3 of Table II (b). The squares of these deviations are entered in column 4, and added, yielding 904·51. (The average value is easily seen to be given by 35+9·5/20=35·475 agreeing with the previous value). The correction is calculated in the same way. Squaring + 9·5 (the sum of deviations) we have 90·25. Dividing this quantity by 20, we get 4·51; subtracting 4·51 from 904·51 we again have 900·00 as the corrected sum of squares, which necessarily agrees with the value found by the more laborious process.

5. The mean value and sum of squares of deviations from the mean values for the other strains are calculated in the same way and entered as shown in Table III. The sum of squares are next divided by 19 to give the estimated † variances which are entered in column 6 of Table III. We shall also require the quantities $S^2/20$, and these are given in column 7 of the same table.

Table III.

Mean values and variances.

(1)	(2) Variety	(3)	(4) Mean value	(5) Sum of squares	(6) S^2	(7) $S^2/20$
1	A	20	35·47	900·00	47·3684	2·36842
2	B	20	47·68	418·75	22·0395	1·10197
3	C	20	45·94	1392·07	73·2205	3·66102
4	D	20	39·25	602·11	31·6895	1·58447
5	E	20	44·09	558·67	29·4037	1·47018

* A recalculation of the standard deviation by the shorter method is in any case desirable as a check on the arithmetic.

† The observed variances (or squares of observed standard deviations) would be given by dividing the sum of squares of deviations by 20, the size of the sample. In Fisher's method it is, however, necessary to use the estimated variances which are obtained by dividing the sum of squares of deviations by the number of degrees of freedom. This number represents the number of independent comparisons possible within the sample, and is given by (N—1) where N is the size of the sample. The estimated variance is the best estimate of the variance of the population from which the sample is drawn.

6. The fundamental formulæ in Fisher's method may be now given. Let m_1 and m_2 be two mean values based on samples of size N_1 and N_2, and let S_1^2, S_2^2 be the two corresponding estimated variances. We then calculate the quantity

$$t = \frac{(m_1 - m_2)}{\sqrt{\frac{S_1^2}{N_1} + \frac{S_2^2}{N_2}}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

When $N_1 = N_2 = N$, we have

$$t = \frac{(m_1 - m_2)\sqrt{N}}{\sqrt{S_1^2 + S_2^2}} \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

In the present case $N_1 = N_2 = 20$, and formula (4) would be slightly more convenient to use. But formula (3) is more general, and as we have already tabulated the values of $S^2/20$ in Table III, column 7, we shall use it in the manner shown below.

Let us compare B and A.

We have—

$m_1 = 47{\cdot}68$, also $S_1^2/20 = 1{\cdot}10197$

$m_2 = 35{\cdot}47$, $S_2^2/20 = 2{\cdot}36842$

Thus $(m_1 - m_2) = 12{\cdot}21$, and $(S_1^2/20) + (S_2^2/20) = 3{\cdot}47039$

From Barlow's Tables we find the square root of 3·47039 to be 1·863. Hence "t" =12·21/1·863=6·554.

In the same way we can calculate the value of "t" for any pair of plants. It is convenient to proceed systematically. We first tabulate the differences between the mean values in Table IV.

TABLE IV.

Differences between mean values.

	A	B	C	D	E
A	..	+12·21	+10·47	+3·78	+8·62
B	—12·21	..	—1·74	—8·43	—3·59
C	—10·47	+1·74	..	—6·69	—1·85
D	—3·78	+8·43	+6·69	..	+4·84
E	—8·62	+3·59	+1·85	—4·84	..

The corresponding values of $(S_1^2/20)$ are then added (for each pair of plants) and entered in Table V.

TABLE V.

Sums of $(S_1^2/n_1+S_2^2/n_2)$.

	A	B	C	D	E
A	..	3·47039	6·02944	3·95289	3·83860
B	3·47039	..	4·76299	2·68644	2·57215
C	6·02944	4·76299	..	5·24549	5·13120
D	3·95289	2·68644	5·24549	..	3·05465
E	3·83860	2·57215	5·13120	3·05465	..

The corresponding square roots are next found from Barlow's Tables, and entered in Table VI.

TABLE VI.

Standard deviations of differences.

	A	B	C	D	E
A	..	1·863	2·455	1·988	1·959
B	1·863	..	2·182	1·639	1·604
C	2·455	2·182	..	2·290	2·265
D	1·988	1·639	2·290	..	1·748
E	1·959	1·604	2·265	1·748	..

Finally the differences $m_1 - m_2$ (given in Table IV) are divided by the corresponding standard deviations (given in Table VI), and entered in Table VII, which thus furnishes the values of "t" corresponding to the comparison of any pair of plants.

TABLE VII.

Values of "t" (n=38).

	A	B	C	D	E
A	..	6·554	4·265	1·901	4·400
B	6·554	..	0·797	5·143	2·238
C	4·265	0·797	..	2·921	0·817
D	1·901	5·143	2·921	..	2·769
E	4·400	2·238	0·817	2·769	..

7. We may now use Fisher's Table IV (p. 139) to test the significance of the observed values of "t". The appropriate value for 'n' in Table IV will be given by (N_1+N_2-2) or 2 (N—1) when the size of each sample is N. In the present case n=(20+20—2)=38. Unfortunately Fisher's Table does not extend beyond n=30. This, however, will not seriously hamper its use as the following examples will clearly show.*

Let us consider the comparison between A and B. Here t=6·554. From Fisher's Table IV we notice that for even n=30, the probability of occurrence of a value of "t" as large as 2·75 is only ·01, that is 1 in 100. The probability of occurrence of a value as great as 6·554 (for n=30, and hence also for n=38) must therefore be less than ·01. Hence we conclude that the observed difference is definitely significant, that is, it is practically certain that B gives a greater "percentage success" than A.

Let us now take B and C. From Table VII, we find t=0·797. Looking up Fisher's Table, we find that for n=∞, the probability of occurrence of a value 't' equal to or greater than 0·797 must be greater than 0·4, *i.e.*, greater than 40 per cent., but less than 50 per cent. That is, even when there is no real difference between the two strains, such a value of 't' as 0·797 will occur no less than between 40 and 50 times in 100 random trials. We conclude, therefore, that on the given data, we cannot assert that B would give better results than C.

8. Proceeding in the same way, we have entered in Table VIII the limiting probability for each comparison.†

TABLE VIII.

Significance of differences : Limits of probability.

	A	B	C	D	E
A	..	<0·01	<0·01	·05 & ·10	<0·01
B	(<0·01)	..	(0·4 & 0·5)	(<0·01)	(·02 & ·05)
C	(<0·01)	0·4 & 0·5	..	(<0·01)	(0·4 & 0·5)
D	(·05 & ·10)	<0·01	<0·01	..	About ·01
E	(<0·01)	·02 & ·05	0·4 & 0·5	(About ·01)	..

* We can, of course, if we so desire extend Fisher's Table IV by interpolation. Let us take the present case for n=38. For P=·01 we notice that for n=30 the value of 't'=2·750 is higher than the value for n=∞ (*i.e.*, for infinitely large samples) t=2·57582, by (2·750—2·57582)=0·17418. We observe that $\frac{8}{30}$=0·8895 approximately. The interpolated difference then is 0·8895×0·17418. Hence the interpolated value of 't' for n=38 will be given by 2·5758+0·1549=2·7307. It is usually sufficient, however, to find the limiting probabilities as shown in the text.

† The brackets in Table VIII correspond to the *minus* sign in the differences given in Table IV. For example, in column 5, the brackets indicate that E gives a lower yield than B and C, while the absence of the bracket indicates that it gives a better yield than A or D.

B, C and E yield significantly greater " percentage success " than A ; while B and C are definitely superior to D. Adopting a slightly lower level of significance, C is seen to be better than D. At a still lower level of significance, we find that B is better than E, but much reliance should not be placed on this result. The difference between D and A or between B and C or between C and E are statistically insignificant.

9. For large samples we can find a numerical value of the probability with the help of a table of the ' Probability Integral '. I have already mentioned that with samples of 20, the probability integral will not give absolutely correct results. The present figures may, however, be used for purposes of illustration.

Let us consider the comparison between B and C. Here t=0·797, this is the same quantity as " x " in Table II of the Tables for Statisticians and Biometricians (edited by Karl Pearson, Cambridge University Press). From this Table II (p. 2), we find :—

For x=0·79, $\frac{1}{2}(1+\alpha)$=0·7852361. The tabulated difference ' Δ ' in $\frac{1}{2}(1+\alpha)$ for a change of+·01 in ' x ' is +·0029085. For a change of (0·797—0·79)=·007 in ' x ', the difference in $\frac{1}{2}(1+\alpha)$ must be $\frac{\cdot 007}{\cdot 01} \times (+\cdot 0029085) = +\cdot 0020359$. We thus have

For x=0·79	$\frac{1}{2}(1+\alpha)$=	0·7852361
	Adding	·0020359
For x=0·797,	$\frac{1}{2}(1+\alpha)$=	0·7872720
Thus,	$\frac{1}{2}(1-\alpha)$=	0·2127280
And	$2\times\frac{1}{2}(1-\alpha)$=	0·4254560
Also	$1-(1-\alpha)$=	0·5745440

Hence if we assume that the observed deviation is as likely to be in excess as in defect, then the probability that we should reach or exceed the observed deviation is given by 0·425456. The probability that we shall not reach or exceed the observed deviation is, therefore, given by 1—0·425456=0·574544. The odds against the observed result are, therefore, roughly 58 to 42. We cannot assert that the observed difference is significant.

It will be remembered that from Fisher's t-table we had concluded by inspection that the probability of occurrence of a value of t as great as 0·797 (for n=38) lies between 0·4 and 0·5. From the probability integral we find that the calculated probability is 0·43. The two results are therefore consistent. (In fact the probability integral will yield fairly reliable results for samples of 20, although such samples cannot be strictly called large samples).

10. It is sometimes necessary to form a rough idea of the size of samples required to yield a significant difference in mean values.* For any given observed

* For example, Mr. Joshi has enquired what is the minimum number of plants for which data should be collected in order to get a significant difference in mean values.

difference in mean values the size of the sample will naturally depend on the variability as measured by the standard deviation.

Let m_1 and m_2 be the two mean values, s_1, s_2 the two estimated standard* deviations based on N plants each from the two strains.

Writing $s = \sqrt{(s_1^2 + s_2^2)/2}$ for the average standard deviation, it is easy to see that

$$t = x = \frac{m_1 - m_2}{s}\sqrt{\frac{N}{2}} \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

Let us write $$f = (m_1 - m_2)/s \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

Then $$t = f\sqrt{\frac{N}{2}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

That is, $$f = t\sqrt{\frac{2}{N}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (8)$$

Also the value of 'n' for entering Fisher's Table IV will be given by $n = 2\,(N-1)$ . . (9)

With the help of the above two equations (7) and (8) it is now easy to calculate the value of 'f' from the corresponding value of 't' given in Fisher's Table IV (p. 139).

TABLE IX.

Values of "f". (Theory of small samples.)

N†	P‡			
	0·10	0·05	0·02	0·01
2	2·920	4·303	6·965	9·925
3	1·761	2·267	3·059	3·759
4	1·374	1·730	2·222	2·621
5	1·176	1·458	1·831	2·122
6	1·046	1·286	1·596	1·829
7	0·952	1·165	1·433	1·633
8	0·881	1·073	1·312	1·488
9	0·823	0·999	1·218	1·377
10	0·775	0·940	1·141	1·287
11	0·736	0·889	1·078	1·213
12	0·701	0·847	1·024	1·151

*That is, the variance is obtained by dividing the sum of squares of deviations by (N—1) the appropriate degrees of freedom.

† N=size of samples.

‡ P=probability of occurrence of 'f'.

TABLE IX—*contd.*

Values of "f". (*Theory of small samples*—contd.)

N*	P†			
	0·10	0·05	0·02	0·01
13	0·671	0·810	0·978	1·097
14	0·645	0·777	0·937	1·050
15	0·621	0·748	0·901	1·009
16	0·600	0·722	0·869	0·972
17	0·581	0·6986	0·8399	0·9395
18	0·564	0·6774	0·8139	0·9098
19	0·548	0·6581	0·7901	0·8828
20	0·5332	0·6403	0·7683	0·8580
21	0·5197	0·6239	0·7482	0·8352
22	0·5072	0·6086	0·7296	0·8141
23	0·4955	0·5944	0·7123	0·7946
24	0·4844	0·5809	0·6958	0·7760
25	0·4745	0·5689	0·6811	0·7593
30	0·4317	0·5170	0·6181	0·6883
35	0·3987	0·4772	0·5699	0·6341
40	0·3723	0·4453	0·5314	0·5909
50	0·3321	0·3970	0·4733	0·5258
75	0·2703	0·3228	0·3842	0·4264
100	0·2337	0·2789	0·3318	0·3680

Such a table of f-values is given in Table IX for 4 different levels of significance; P=0·10, 0·05, 0·02 and 0·01. In this table 'N' represents the size of each of the two samples on which the two mean values to be compared are based, and 'f' gives the largest value of $\frac{(m_1-m_2)}{s}$ which is significant within the assigned degree of probability (10 per cent., 5 per cent., 2 per cent. or 1 per cent.).

*N=size of samples. †P=probability of occurrence of 'f'.

From N=2 to N=16, the 'f'=values are calculated directly from the 't'= values given in Fisher's Table IV. From N=17 to N=100, they are obtained from interpolated values of 't' in the same table.

11. If "s" is known even approximately, it is possible with the help of the above Table IX to find roughly the value of N, the size of the samples required to make any observed difference in mean values statistically significant.

For example, for Mr. Joshi's data, we can find an average value of (s^2) by adding the different values of sums of squares given in Table III, for the 5 different strains and dividing by 100. The average variance comes out to be 40·74 leading to an average value of s=6·4 approximately.

We can now use this value of s=6·4, in conjunction with Table IX, to give a rough idea of the size of the samples required for specific purposes.

Example (*i*). Let us compare B and A. The observed difference is 12·21 in favour of B. Dividing 12·21 by 6·4, we get f=1·91 approximately. Adopting P=·01, (that is odds of 100 to 1), we notice from Table IX that for N=6, 'f' is 1·829. We conclude that for this particular comparison, the observed difference would have been significant even if the mean values were based on small samples of size 6 or 7.

Ex. (*ii*). For C and D the observed difference is 6·69. That is f=1·05. In Table IX, with P=·01, and N=14, 'f' is 1·05. In this case samples of size 14 would be sufficient.

Ex. (*iii*). For D and A the observed difference is 3·78, giving a value f=0·59. For P=·01, N=40, f is 0·59 in Table IX. With odds of 100 to 1, we shall therefore require samples of size 40. Working with a 5 per cent. probability, we notice that N=23 gives a value of f=·5944. Hence with odds of 20 to 1, samples of size 23 would be just sufficient.

Ex. (*iv*). For B and C the observed difference is 1·74, with f=·272. With 5 per cent. probability we find that f=·2789 for N=100. We thus conclude that samples of size greater than 100 must be collected in order to make this particulars observed difference statistically significant.

I need scarcely note that unless we have some idea (even if rough and tentative) regarding the magnitude of the standard deviation, it is not possible to say anything about the required size of the sample.

Although in the above discussion the size of the sample has been assumed to be equal for both varieties, it is not necessary that this should be so in actual practice. Fisher's formula is quite general, and would apply for the comparison of mean values for two samples when the size of the sample is different in the two cases. It should

be remembered that in using Fisher's Table IV, n should be taken equal to (N_1+N_2-1), and we must write the t=formula as :—

$$t=f\sqrt{\frac{N_1 \cdot N_2}{N_1+N_2}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7,1)$$

$$\text{and } f=t\sqrt{\frac{N_1+N_2}{N_1 \cdot N}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (8,1)$$

TABLE X.

Values of "f". (Theory of large samples.)

N*	P†		N*	P†	
..	0·05	0·01	..	0·05	0·01
2	1·9600	2·5758	17	0·6723	0·8835
3	1·6003	2·1032	18	0·6533	0·8586
4	1·3859	1·8214	19	0·6359	0·8357
5	1·2396	1·6291	20	0·6198	0·8146
6	1·1316	1·4872	21	0·6049	0·7949
7	1·0476	1·3768	22	0·5909	0·7766
8	0·9800	1·2879	23	0·5779	0·7593
9	0·9240	1·2142	24	0·5655	0·7432
10	0·8765	1·1519	25	0·5544	0·7286
11	0·8357	1·0983	30	0·5060	0·6651
12	0·8001	1·0514	35	0·4685	0·6157
13	0·7689	1·0105	40	0·4382	0·5760
14	0·7409	0·9737	50	0·3920	0·5162
15	0·7157	0·9406	75	0·3201	0·4206
16	0·6929	0·9107	100	0·2772	0·3643

12. In this connexion it will not be out of place to say a few words regarding the magnitude of the inaccuracy involved in the use of the theory of large samples. In Table X will be found the values of 'f' calculated on the basis of the theory of large samples. For N=2, an observed value of f=2·58 would be considered significant (with P=·01) on the classical theory, whereas using the theory of small

* N=size of samples.

† P=probability of occurrence of 'f'.

samples we notice that 'f' must reach the value of 9·93 for samples of 2 before the observed difference can be asserted to be significant with the same degree of probability.

It will be noticed that the difference between 'f' values in the two Tables IX and X at first decreases rapidly with the increase of N, and then decreases more gradually. For N=10, the difference in the value of 'f' is about 11 per cent. (P=·01). For N=25, the difference is just over 4 per cent., while for N=100, the difference is just about 1 per cent. The theory of large samples may, therefore, be used with safety for N greater than 100. It may be used without introducing appreciable errors practically for samples of size greater than 25.

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CALCULATION OF PROBABLE ERROR OF MENDELIAN RATIOS

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In most of the crop improvement programmes it is very often necessary to assemble in one or two varieties some of the more important agronomic and commercial characters. This is accomplished by having recourse to the process of hybridisation. In carrying out such investigations, it is essential to know the mode of inheritance of the useful characters; in what manner they segregate in later progenies; how combinations and recombinations take place; the interaction of factors, linkage, and crossing over, etc., so that in assembling the desired factors, crosses can be intelligently made so as to derive the maximum benefit.

In field experimentation it is very rarely possible due to errors of random sampling, soil heterogeneity, production of functional gametes in unequal numbers, selective mating, etc., to obtain perfect fits to the various Mendelian frequencies and interpretation of results in consequence is extremely difficult. Much of the difficulty is got over by applying statistical methods to analyse the genetic data so that the probable mode of inheritance of factors can be properly studied.

The text books on Genetics by Babcock and Clausen, Castle, Crew, Sinnott and Dunn, and Walter have dealt with certain aspects of statistical methods, but the methods of calculating the probable errors of Mendelian results are not to be found in their books and they still lie scattered in journal articles and experiment station bulletins, many of them not easily accessible. This paper has been written with a view to bring these methods to the notice of Indian plant-breeders. It is as well to mention that in the Genetical Laboratories of the more important colleges and universities in the U. S. A., much of the material here given is in the form of cyclostyled sheets as laboratory helps to the students of Genetics.

When two plants that possess contrasting pairs of factors are hybridised, the factors begin to segregate in the F_2 generation. The investigator then divides the progeny into the phenotypic classes and the observed numbers in each class are compared with the expected frequency which may be 3 : 1, 9 : 7, 9 : 3 : 3 : 1, or 27 : 37. As already stated the observed numbers rarely fit the expected ones and a goodness-of-fit is obtained for seeing how far the deviations are or are not significant. This is

done by dividing the deviations with their respective probable errors and then finding whether the odds are or are not in favour of the hypothesis.

The procedure followed will become clear from a concrete case. In an experiment to see the mode of inheritance of *Fusarium* wilt resistance in canning peas, Wade [1930] observed that in the F_2 progeny there were 335 plants which segregated as 264 healthy and 71 diseased. If segregation was on the basis of a 3 : 1 ratio, then $\frac{3}{4}$ of the total or 251·25 plants would have been healthy and $\frac{1}{4}$ of the total or 83·75 plants would have been diseased. If it was a case of 9 : 7 ratio, then $\frac{9}{16}$ths of the total or 188 plants would have been healthy and $\frac{7}{16}$ths of the total or 147 would have been diseased. Wade concluded that the segregation agreed with a 3 : 1 ratio with resistance as the dominant factor.

The observed figures deviate from the calculated ones by (83·75—71)=12·75. It is now necessary to see if that is a significant deviation or an insignificant one due to errors of random sampling and other causes.

The probable error of Mendelian ratios can be determined in three ways: the method of absolute numbers, the method of percentages, and the method of ratios. The method commonly used by geneticists is the method of absolute numbers and that method is described below.

The method of absolute numbers

The standard deviation of a binomial distribution is $\sqrt{p \cdot q \cdot n}$ where p and q are the elements of a ratio, expressed in decimals, and n the number of individuals in the experiment. The probable error is obtained by multiplying the standard deviation with the constant, ·6745.

Probable error $=\cdot 6745\sqrt{p \cdot q \cdot n}$ and $p+q$ is unity.

In the case of a 3 : 1 ratio, p is $\frac{3}{4}$ or ·75 and q is equal to 1—·75 or ·25.

$$\text{P. E.}_{3:1}=\cdot 6745\sqrt{\cdot 75\times \cdot 25\times n}=\cdot 292\sqrt{n}$$

In the case of a 27 : 37 ratio, for instance, $p=27/64=\cdot 422$ and $q=(1-\cdot 422)=\cdot 578$.

$$\text{P. E.}_{27:37}=\cdot 6745\sqrt{\cdot 422\times \cdot 578\times n}=\cdot 333\sqrt{n}$$

Deviation is determined by noting the difference between observed and calculated frequencies. The goodness-of-fit of Wade's data may now be found:

	Healthy	Diseased
Observed	264	71
Calculated on 3 : 1 basis.	251·25	83·75

Deviation = 12·75 and n = 335

$$\text{P. E.}_{3:1}=\cdot 292\sqrt{335}=5\cdot 35$$

$$\frac{\text{Deviation}}{\text{P. E.}}=2\cdot 4$$

The quotient obtained on dividing the deviation by the probable error is 2·4. Pearl and Miner [1914] have published a table showing the probability of occurrence of statistical deviations of different magnitudes relative to the probable error. The table will also be found in the text by Hayes and Garber and the Laboratory Manual of Genetics by Babcock and Collins. From the table the odds against the occurrence of a deviation as great or greater than the designated one are obtained, which show whether the data fit the hypothesis. In the example the value 2·4 has been obtained and the odds are 8·48 to 1. Geneticists have accepted that when the value of the ratio, $\frac{\text{deviation}}{\text{probable error}}$, is three or less than three, then the odds are in favour of the experimental data fitting the assumed hypothesis, provided that the value of n is not very small. When the value is over three then the investigator has to be sceptical of his data and repeat the experiment or see if they fit some other ratio. In general it may be stated that smaller the deviation and larger the probable error better the data fit into the hypothesis.

Probable error formulæ for various Mendelian ratios have been calculated and are given below:

$$\text{P.E.}_{3:1} = \cdot6745\sqrt{\cdot75\times\cdot25\times n} = \cdot292\sqrt{n}$$

$$\text{P.E.}_{1:1} = \cdot6745\sqrt{\cdot50\times\cdot50\times n} = \cdot337\sqrt{n}$$

$$\text{P.E.}_{9:7} = \cdot6745\sqrt{\cdot5625\times\cdot4375\times n} = \cdot335\sqrt{n}$$

$$\text{P.E.}_{15:1} = \cdot6745\sqrt{\cdot9375\times\cdot0625\times n} = \cdot163\sqrt{n}$$

$$\text{P.E.}_{3:13} = \cdot6745\sqrt{\cdot1875\times\cdot8125\times n} = \cdot263\sqrt{n}$$

$$\text{P.E.}_{10:6} = \cdot6745\sqrt{\cdot625\times\cdot375\times n} = \cdot326\sqrt{n}$$

$$\text{P.E.}_{27:37} = \cdot6745\sqrt{\cdot422\times\cdot578\times n} = \cdot333\sqrt{n}$$

For any two distributions other than the above, such as 63:1, etc., it will be noted how simple it is to calculate the probable error.

For Mendelian frequencies of more than two distributions such as 1:2:1, 9:3:3:1, the above method of finding the goodness-of-test can also be applied. In the former case, the first two frequencies are added together and the probable error on the basis of the ratio 3:1 is obtained. By dividing the deviation by the probable error the significance or insignificance of the deviates is obtained.

When segregation is dihybrid, of 9:3:3:1, the method adopted is to add the extreme classes, (9 + 1), and the mean classes, (3 + 3). The goodness-of-fit is then found by seeing the deviation from an expected ratio of 10:6 and by dividing the deviation with the probable error. The following example taken from Carver [1929] will make the calculation clear.

Segregation was as follows in a particular cross handled by him :—

Naked seeded red plants	= 68		
Fuzzy seeded red plants	= 20		
Naked seeded green plants	= 24		
Fuzzy seeded green plants	= 12		
			Calculated on 10:6.
Sum of extreme classes = 68 + 12	=	80	77·5
Sum of mean classes = 20 + 24	=	44	46·5
Deviation = 2·5 P. E. $_{10:6}$ = 3·6. Dev./P. E. . . .	=		0·6

The methods suggested above are simple and when ' n ' is sufficiently large it is possible to evolve a fairly correct hypothesis on the manner of inheritance of the characters and establish the number of genetic factors involved. But sometimes it so happens that the number of individuals in each progeny row or family is very small. The segregation in each one of these families may deviate from the expected frequency considerably but when the numbers in each phenotypic class are summated, the deviations from expectation in the individuals may not become evident. Total class frequencies so obtained by adding are therefore composite values and as such may mask a serious lack of consistency in the numerical ratios of the separate families.

Kirk and Immer [1928], who have drawn attention to this, have given a good illustration to prove how summating the phenotypic classes and then comparing the totals so obtained with the expected numbers lead to erroneous conclusions. In the case they have given the total of the dominants and the recessives respectively agreed with a 3:1 Mendelian segregation. But the number of individuals in each family was too small and some of the segregations in the families were significantly different from expectations.

In such cases, it is a good practice to apply the goodness-of-fit test outlined above to each family segregation. If significant deviations are discovered, then more data for further substantiation of the hypothesis must be sought for. Kirk and Immer have suggested the application of the Chi squared (X^2) test in such cases.

It was stated above that for Mendelian frequencies like 1:2:1 or 9:3:3:1 the goodness-of-fit test described in this paper could be applied. However for these ratios the method is supposed to be crude and the application of the X^2 test to be more correct. The X^2 distribution was first suggested by Pearson and a table giving the values of P was prepared by Elderton in 1900. The application of this test of agreement to Mendelian polyhybrid ratios of single segregating progenies was suggested by Harris [1912]. The method is outlined both by Harris, and Fisher [1930] who has also given a table for the values of P in a more convenient form.

The paper may be concluded with a quotation from Kirk and Immer ; " While refined statistical analysis is an aid in determining the validity of a genetic hypo-

thesis, it must be admitted that an adequate genetic proof of a hypothesis seldom can be found on the basis of F_2 data alone. The back cross method or the growing of the progeny of selected F_2 plants in F_3 is necessary to prove the number and nature of genetic factors involved."

REFERENCES.

Carver (1929). *Jour. Amer. Soc. Agron.* **21**, 467—480.
Fisher (1930). Statistical Methods for Research Workers, Edinburgh.
Harris (1912). *Amer. Naturalist* **46**, 741—745.
Kirk and Immer (1928). *Scientific Agriculturist* **8**, 745—750.
Pearl and Miner (1914). *Maine Agric. Expt. Stat. Bull.* 226.
Wade (1930). *Wis. Agric. Expt. Stat. Res. Bull.* 97.

OBSERVATIONS ON MALE NUCLEI IN THE SUGARCANE

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(With Plates IX-XI.)

I. INTRODUCTION.

The germination of sugarcane pollen in artificial media has, of late, been extensively employed at the Imperial Sugarcane Breeding Station, Coimbatore, for ascertaining the male fertility of the varieties used in crossing. Fairly high percentages of germination have been obtained by this method in certain of the thick and thin types of canes [Dutt and Ayyar, 1928], as also in *Saccharum spontaneum* and *S. Narenga*. In some cultures the pollen tubes were long enough to reach the ovary if they were growing on the style of the plant [Dutt, 1929]. The artificial culture method has thus been quite successful in the percentages of germination and in the length attained by the pollen tubes; attempts were therefore made to see if the migration of the male nuclei takes place *in vitro*. For staining, Belling's [1921] aceto-carmine was used as this stain has been successfully employed by Brink [1924].

II. PRESENCE OF TWO MALE NUCLEI IN THE POLLEN GRAIN BEFORE SHEDDING.

The freshly shed pollen grains of the sugarcane varieties—Kaludai Boothan, Co. 213, Co. 285, D. 74, D. 131, D. 1135, E.K. 28, S.W. 111, P.O.J. 2696, P.O.J. 2878, Ges. Preanger, Maur. 131, N.G. 24 and *Saccharum spontaneum* (local and

Glaggah) were stained and were in all cases found to contain two male nuclei and one tube nucleus. The tube nucleus took the stain rather faintly, while the male nuclei were deeply stained.

To ascertain if the division of the generative nucleus takes place earlier than the time of the dehiscence of the anther, such flowers were taken as would open the next morning. The anthers were removed and teased in aceto-carmine. In all the varieties thus examined, two male nuclei were clearly stained. This would indicate that the generative nucleus divides into two male nuclei at least 24 hours before shedding. In Plate IX, fig. *g*, is shown a fairly early stage where the nucleus has not yet divided into tube and generative nuclei.

The shape of the male nuclei in the pollen grain varied from roughly spherical to spindle or crescent-shaped as shown in Plate IX, figs. *a* to *f*.

III. Migration of the nuclei into the pollen tube.

Different stages in the migration of the nuclei into the pollen tube were observed. In Plate X, fig. *a*, the pollen tube has been formed, but the nuclei have not yet migrated. A similar stage is shown in Plate X, fig. *b*, but on a stigmatic papilla. The migration has taken place in figs. *c* and *d*, but in the latter the tube nucleus has not been stained.

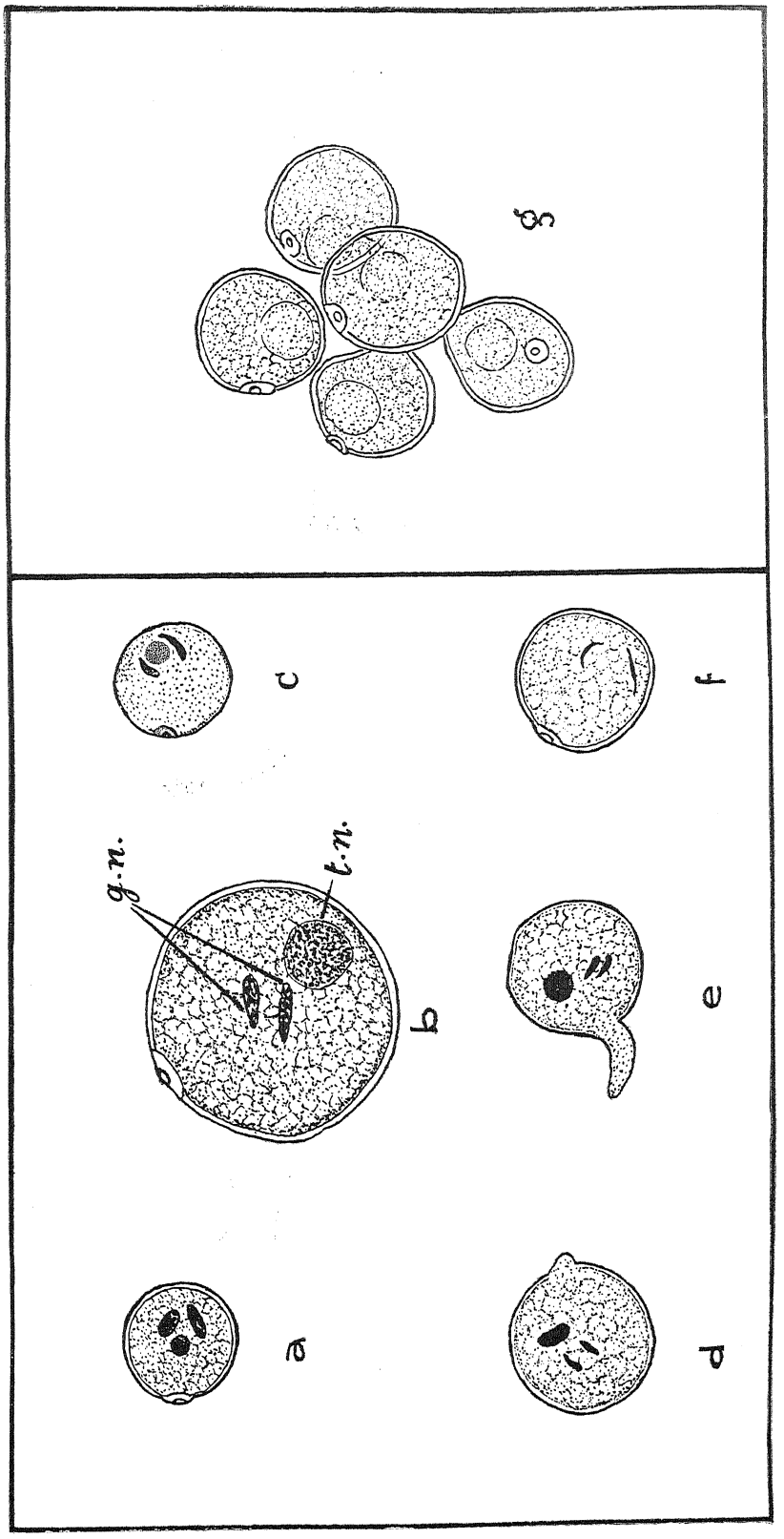
In several cultures, pollen grains were met with which had not germinated, but in which the nuclei were apparently normal. Brink [1924] records a similar experience in *Scilla*.

The male nuclei in the pollen tubes were mostly elongated in shape. In certain cases they were more or less spherical and in others somewhat spirally curved.

Four male nuclei were noticed in certain pollen tubes and in a few pollen grains of the variety B. 3412, C. A. C. 87 and P. O. J.1410. The different positions occupied by these nuclei are shown in Plate XI, figs. *b* to *e*. All the four nuclei stained deeply and were therefore most probably male nuclei. The tube nucleus was not clearly stained in either of these. The pollen grain in Plate XI, fig. *a*, however, shows the tube nucleus and four male nuclei. The shape of the nuclei in the pollen tube varied, as seen in Plate XI, figs. *b* to *e* from more or less spherical to somewhat vermiform.

IV. Discussion of results.

The division of the generative nucleus into two male nuclei inside the pollen grain prior to shedding, which was observed in all the fourteen sugarcane varieties


a
b
g.n.
t.n.
c
d
e
f
g



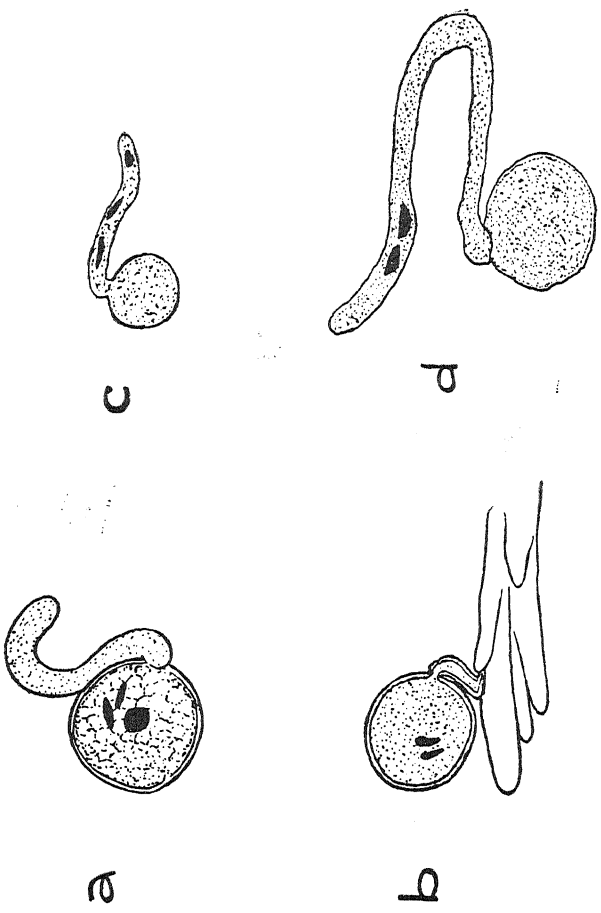
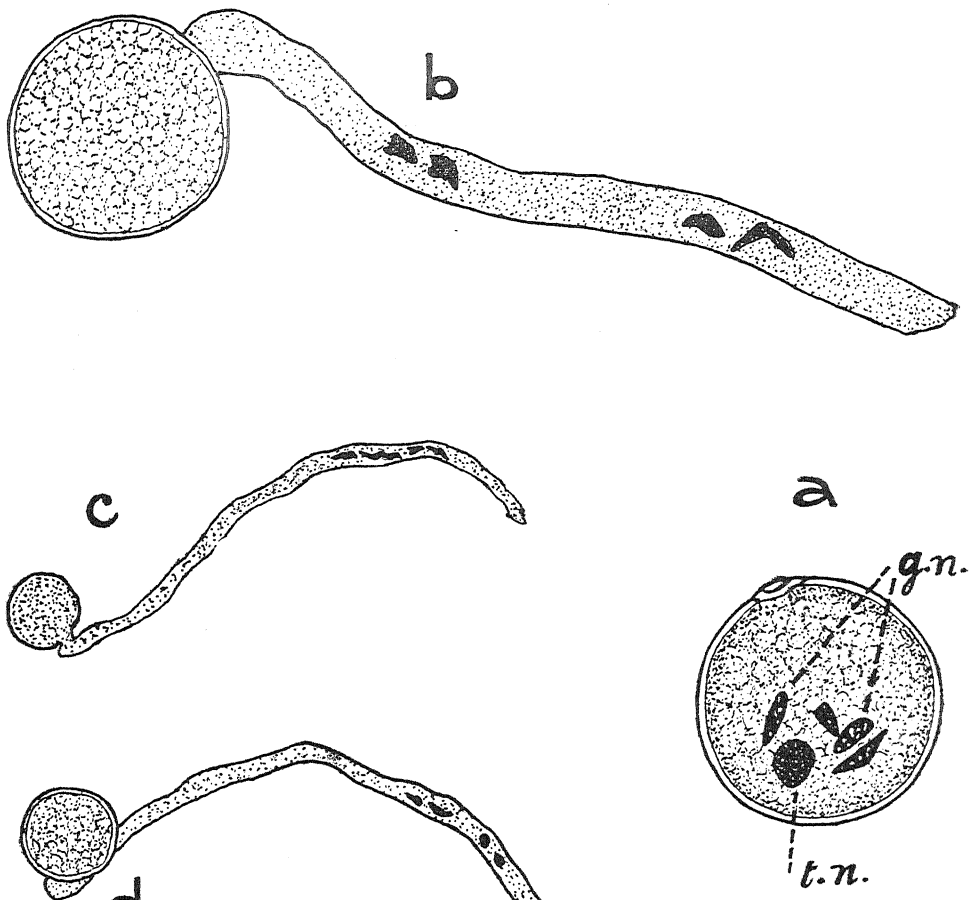

a
b
c
d

PLATE XI.



studied and in *Saccharum spontaneum*, would appear to be characteristic of quite a number of grasses, as this condition was met with by Golinski [1893] in *Triticum* and other grasses, and by Cannon [1900] in *Avena fatua*. Weatherwax [1923] noticed in maize that the mature pollen grain at the time of leaving the anther contains fully developed sperms, which are two small crescent-shaped cells with long attenuate ends. Percival [1921] mentions that the pollen grains of wheat germinate while still in the un-opened anther and possess two slender male gametes each of which is curved and pointed at one end. Artschwager, Brandes and Starrett [1929] have figured two male nuclei in the mature pollen grain of the sugarcane variety U.S. 1694.

In the sugarcane varieties studied by us, the form of the male nuclei in the pollen grain was not constant. It ranged from spherical to crescent or spindle-shaped. As the male nuclei migrated into the pollen tubes, they sometimes retained the spherical shape, but mostly became elongated. In a few instances they were observed to be spirally curved, but this shape was noticed only inside the pollen tubes and on no occasion inside the pollen grain.

The occurrence of four male nuclei is rather unusual though not unprecedented, as Coulter and Chamberlain [1909] mention this condition as having been observed by Strasburger [1884] as sometimes occurring in *Camassia Fraseri*, and Chauveaud [1892] found four or five bodies in the pollen tubes of *Vincetoxicum nigrum* and *V. medium*, which he thought might be interpreted as male nuclei and responsible for polyembryony. The four male nuclei have so far been observed by us only in three sugarcane varieties and the phenomenon will need to be studied more thoroughly before it can be said what its real significance is, but most probably as pointed out by Coulter and Chamberlain, it may have no further significance than that any active cell may be induced to divide by favourable conditions.

REFERENCES.

Artschwager, Brandes and Starrett (1929). *Jour. Agric. Res.* **39**, 11.
Belling (1921). *Amer. Nat.* **55**, 572-573.
Brink (1924), *Amer. Jour. Bot.* **11**, 351-364.
Cannon (1900). *Proc. Calif. Acad. Sci.* **1**, 329-364.
Chauveaud (1892). *Compt. Rend.* **114**, 504-506.
Coulter and Chamberlain (1909). Morphology of Angiosperms, 136-138, 217.
Dutt and Ayyar (1928). *Agric. Jour. India* **23**, 190-200.
Dutt (1929). *Agric. Jour. India* **24**, 235-244.
Golinski (1893). *Bot. Central Bl.* **55**, 1-17.
Percival (1921). The Wheat Plant, p. 119.
Strasburger (1884). Neue Untersuch. uber den befracht den Phanerogamen.
Weatherwax (1923). The Story of the Maize Plant, p. 134.

EXPLANATION OF PLATES.

All the drawings have been made with a Camera Lucida, and at the level of the stage.

Plate IX. Figures *a* to *f* show the shape of the male nuclei in the pollen grains. Fig. *g* is an early stage of the male gametophyte where the nucleus has not yet divided into the tube and generative nuclei. The magnification of fig. *b* is 770 diameters; all other figures 380 diameters.

a. P.O.J. 2878 pollen grain, 24 hours before shedding.

b. P.O.J. 2696 pollen grain, 24 hours before shedding.

t. n. tube nucleus.

g. n. generative nuclei.

c. Co. 285 pollen grain just before shedding.

d & e. B. 3412 pollen grains growing *in vitro*.

f. Co. 285 pollen grain just before shedding.

g. Co. 243 pollen grains from an undehisced anther.

Plate X. Various stages in the migration of the male nuclei in the pollen tubes.

a. × 380. *Saccharum spontaneum* pollen *in vitro*.

b. × 380. *Saccharum spontaneum* pollen germinating on a stigmatic papilla.

c. × 240. B. 3412 pollen *in vitro*.

d × 380. Co. 285 pollen *in vitro*.

Plate XI. Four male nuclei in the pollen grain and pollen tubes of the variety B. 3412.

a. × 770. t. n. tube nucleus.

g. n. generative nuclei.

b. × 770. Four male nuclei in two pairs.

c. × 240. Two of the nuclei are somewhat spirally curved.

d. & e. × 240. Male nuclei in pairs. Note the relative distance between the first and the second pair of nuclei.

PLATE XII.

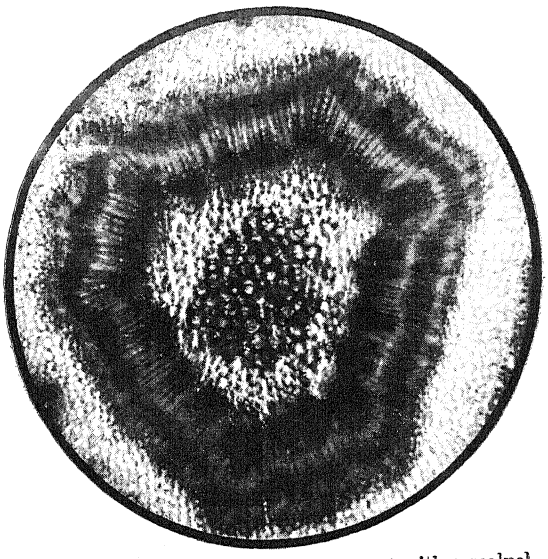


Fig. 1. A thick section of cotton-stem cut with a scalpel and mounted in water.

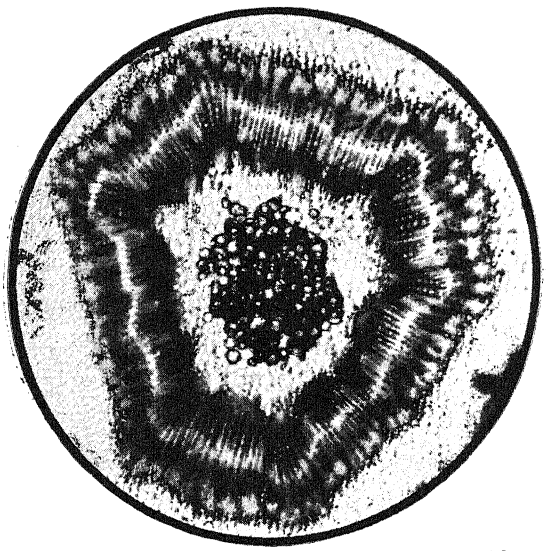


Fig. 2. The same section mounted in lactic acid (the photographic elements kept constant for both).

A NOTE ON THE USE OF LACTIC ACID IN PLANT HISTOLOGY.*

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(With Plate XII.)

In connection with the study of the nature of some plant-tissues under the microscope, various organic acids were used on thick sections, cut with a scalpel. When such sections which were too thick to be seen through, were mounted in concentrated lactic acid, there was a remarkably quick clearing action, and the parts became as clearly visible as in very thin sections cut carefully with a razor. On similar sections of the cotton stem, the reagent brought out the structure of the vascular bundles and the cambium clearly; the accompanying micro-photographs are only approximate illustrations of the striking effect produced by lactic acid. Later, this was found to be very valuable in studying sections of hard seed coats of which thin sections could not be easily secured. The method was tested with a variety of materials, such as, (1) mucilaginous tissues:—Cotton stem, pedicel, seed coat, and cotyledon, (2) resinous tissues:—Margosa (Neem) stem and petiole and (3) nitrogenous tissues:—Bengal gram stem and seed. In all cases the clearing effect was produced very well. Other organic acids such as acetic, citric, malic, and tartaric acids or their combinations with glycerine were not found to be of such use.

A partial explanation of the value of lactic acid will probably be that it acts as clearing agent by its strongly refractive property and that it is also a mild macerating agent, performing the same function as oxalic, tartaric and acetic acids. Its advantages are (1) the reaction is quick and it produces its effect in the cold and does not require heating; (2) unlike the alkalis and chloral hydrate, which clear by destroying or swelling, this acts by equalizing the refractive differences; (3) its miscibility with water renders mounting simple; (4) its use for staining purposes with cotton-blue is well known; (5) it excludes air from inter-cellular spaces pretty

* The use of lactic acid as a reagent for clearing microscopical preparations of plant tissues is not altogether unknown for angiosperms, as it has been mentioned as a specially good reagent for sections from herbarium material, etc. Its use, however, is not common in Indian laboratories and the method deserves to be brought to the notice of the Indian workers.—EDITOR.

completely; and (6) sections can be preserved in it for a considerable time. The following table gives a comparison of some of the properties of lactic acid with those of glycerine.

	Glycerine	Lactic acid
1 Density	1·26	1·25
2 Refractive index . . .	1·47	1·44
3 Viscosity	Thick syrup	Thick syrup
4 Miscibility with water . .	Full	Full

Besides having all the physical properties of glycerine, lactic acid has in addition, the advantages arising from its acid characters. Thus it not only secures the best preservative attainable of normal conditions, but also offers a simplified and ready method, which renders possible rapid and increased accumulation of data.

Though lactic acid has been employed as a component of lactophenol (in which it is mixed with phenol, cotton-blue, glycerine and water) and also used in studying mycelia, its utility in so greatly simplifying histological technique seems not to have been adequately recongnised.

FISHER'S ANALYSIS OF VARIANCE WITH PADDY ON A FIELD-SCALE

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(Received for publication on the 28th September 1931.)

(With five text-figures.)

I. INTRODUCTORY.

For some years prior to 1929—in fact, since proper statistical treatment had been given—experiments on the College Farm at Mandalay had been based on some form of Student's Method. Each plot of each treatment had been compared with an adjacent control or with the average of two adjacent controls. This method, while no doubt giving very valid results, Lord [1924] stated "that with six replications the probable error of the difference of two plots can be reduced to 2 per cent. by Student's Method and below that figure by that method modified", when combined with the desired number of replications such as five or six was very exhaustive in land, labour, supervision and money for a very limited questionnaire. Thus a straight comparison of treatments A B D E against Control C with six replications (Fig. 1) would require fifty-four plots all requiring separate lay-out, treatment, planting, attention, harvesting, threshing, weighing, etc.

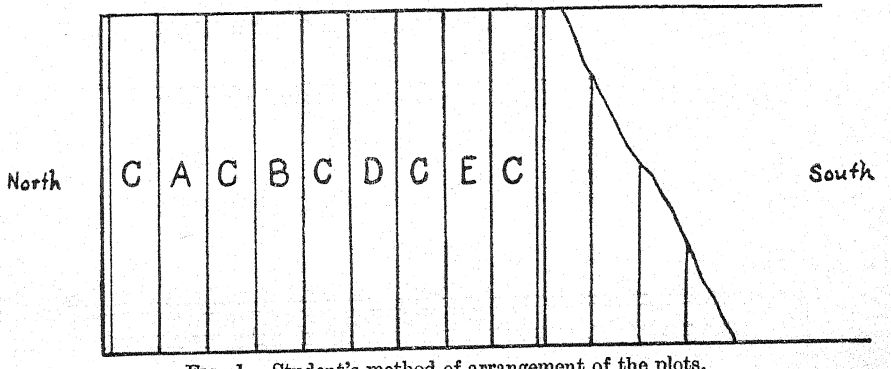


FIG. 1.—Student's method of arrangement of the plots.

It was therefore with great interest that the writer read E. J. Maskell's [1929] articles in *Tropical Agriculture* in 1929 on Experimental Error, explaining Fisher's

Method combining the Randomised Block or Latin Square design with the Analysis of Variance Statistical Interpretation. This seemed to offer many advantages from the point of view of economy and an enlarged questionnaire. Even if the results could not be quite so precise as with Student's modified method (treatment compared with average of two adjacent controls), the extra precision might not be worth the extra trouble and expense. Accordingly it was decided to try out the new method on a new experiment being put down that year. But here before describing the experiment it is advisable to give some explanation of the agricultural conditions.

The paddy land of the Farm is irrigated and is laid out in half acre plots (172′×123′) lying along strips between irrigation channels and drains laid out at right angles to the Main Farm Irrigation Channel (Fig. 2).

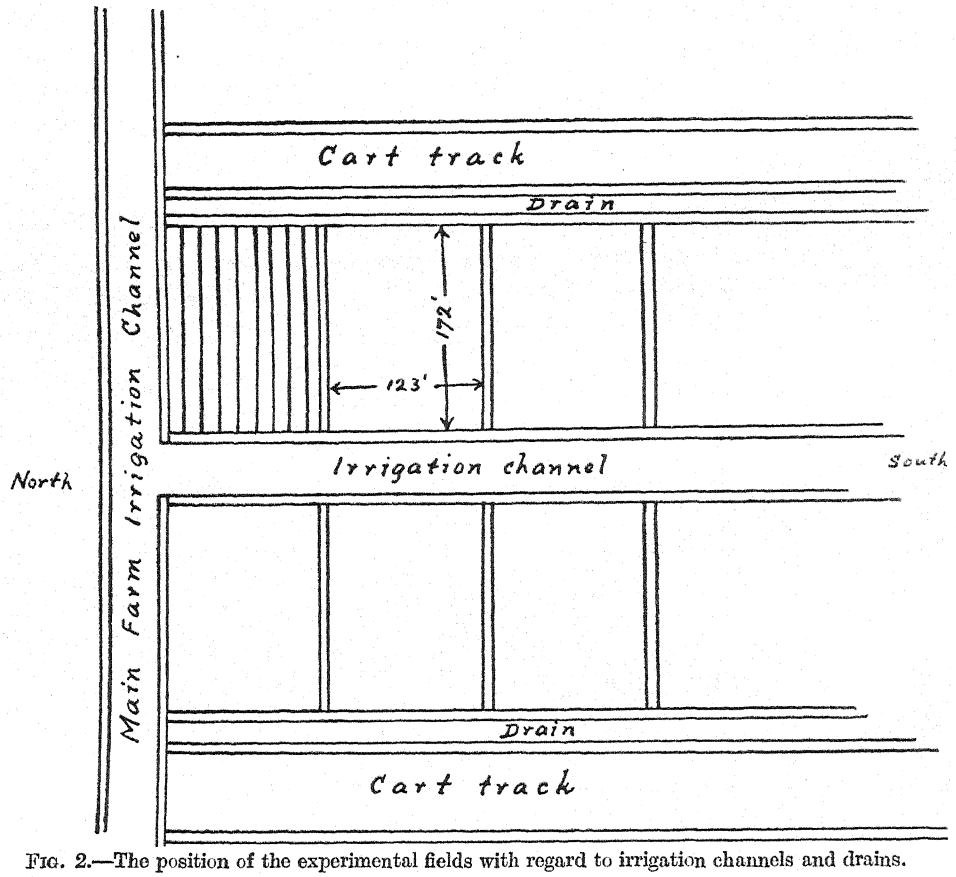


FIG. 2.—The position of the experimental fields with regard to irrigation channels and drains.

It was further known that fertility tended to decrease from irrigation channel to drain within fields. Thus it was very sound experimentally and very suitable

practically to divide the half-acre fields up into long plots elongated in the direction of the fertility gradient within fields and also to combine these plots into blocks running across it [Christidis, 1931]. Unfortunately there was also a general fertility gradient from north to south, *i.e.*, away from Main Irrigation Channel to higher land, which had to be left entirely to the block and random arrangement to allow for. There were no other obvious sources of error and the history sheet of the fields was clean for three years back. The paddy is treated as swamp paddy throughout, *i.e.*, under anaerobic conditions with puddled soil and standing water.

II. The Experiment.

(*a*) *Object.*—The Law of Diminishing Returns seemed to act very early and severely in the case of the new compound ammonium-phosphatic fertilisers just introduced and found successful on paddy. It was therefore decided to test if the limiting factor was organic matter and apply graded doses of F. Y. M. in conjunction with diammonphos 20-50 grade, *i.e.*, a higher proportion of P_2O_5. However the object of the experiment has very little bearing on the subject.

(*b*) *Treatments.*—There were five, *viz.* :—

(1) Control (or no manure).

(2) Diammonphos at the rate of 20 lbs. N_2 per acre.

(3) Diammonphos at the rate of 20 lbs. N_2+1 ton F. Y. M. per acre.

(4) Diammonphos at the rate of 20 lbs. N_2+2 tons F. Y. M. per acre.

(5) Diammonphos at the rate of 20 lbs. N_2+3 tons F. Y. M. per acre.

(*c*) *Design.*—Three half-acre fields size 172′×123′ were available and it was decided to lay the experiment out according to the Randomised Block method as being the best way to utilise the area and shape of the land available. By having six blocks (two in each field) of five plots each, six replications would be obtained. The fields were therefore divided up into ten plots size 172′×8′ with a double-bunded drain (Fig. 3) between each pair of plots and a planted space of approximately 4′ against each dividing field bund. The former is used in all manurial experiments here, to reduce to a minimum the chance of passage of fertilising ingredients from one plot to the other in the water through crab holes, etc., and the latter to avoid bund effect. The final lay-out of one representative field is shown in fig. 4.

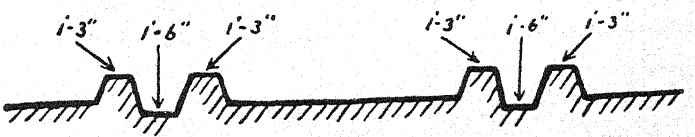


Fig. 3.—Vertical section of plots showing double bunds and drains between them.

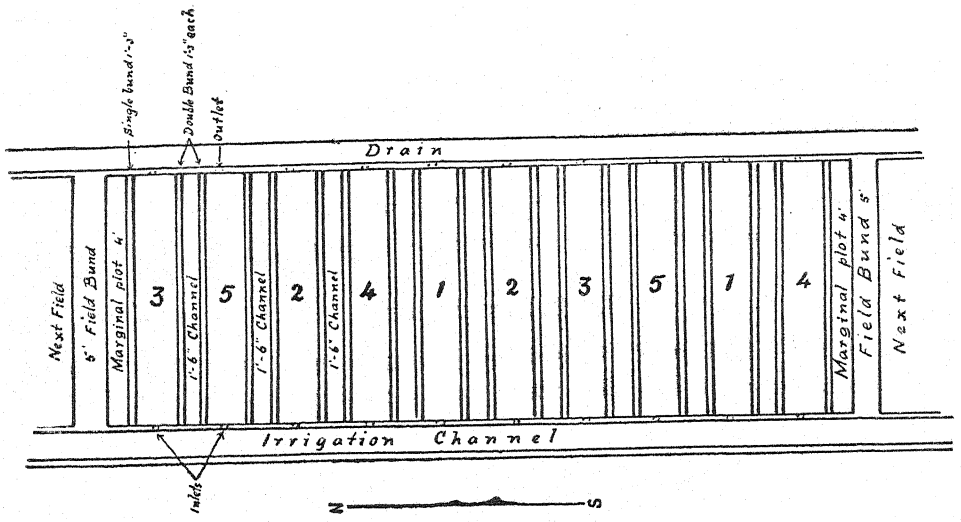


FIG 4.—The final lay-out of one representative field.

Narrower plots than this would cause difficulty in turning bullocks with the harrow and in fact a width of 9′ would be better. The ratio $\frac{\text{length}}{\text{width}}$ of each plot after reducing length to 165′ is over 20:1 and the area is $\frac{1}{33}$ of an acre.

Finally the treatments were allotted to each block in turn at random [Fisher 1930, 1]. One or two blocks had to be repeated to avoid a systematic arrangement and obtain a good distribution before the actual arrangement (Fig. 5) was procured. It should be noted that the total number of plots is only thirty.

North																													South
	3	5	2	4	1	2	3	5	1	4	5	4	2	3	1	3	5	1	4	2	1	2	4	5	3	4	1	3	2	5	
	(359)	(333)	(301)	(305)	(225)	(273)	(294)	(311)	(212)	(303)	(348)	(313)	(290)	(287)	(218)	(297)	(328)	(213)	(309)	(261)	(221)	(305)	(310)	(308)	(243)	(276)	(200)	(277)	(273)	(304)	

FIG. 5.—Arrangement and yield of plots in $\frac{1}{4}$ lbs.

(*d*) *Application of manures.*—This requires great care to avoid mistakes and to effect even distribution within plots. They were applied one day prior to transplanting after the water had been reduced to a minimum and worked into the soil by one harrowing and one levelling.

(*e*) *Transplanting.*—To randomise the variation in planting, each block was planted separately by the same gang of women, arranged in the same order throughout, *i.e.*, a gang of ten women, two to each plot.

(*f*) *Growth.*—The experiment during growth presented an ideal appearance. The growth of every plot was uniform and within blocks the differences in growth between plots were of the same order in all.

(*g*) *Harvesting.*—Only an exact length of 165′ was harvested from each plot and was done block by block as in transplanting. Drying was given equally.

(*h*) *Yields.*—Yields of plots in $\frac{1}{4}$ lbs. are shown in fig. 5.

III.—Statistical interpretation of results.

Following Fisher's method [1930, 1] and taking the working mean to be zero the following final stages were obtained.

(*a*) *Analysis of Variance.*—

Table I.

Analysis of variance.

Due to	Degrees of freedom	Sum of squares	Variance	$\frac{1}{2}$ loge Variance
Blocks . . .	5	4375·77	875·15	..
Treatments . . .	4	39909·54	9977·39	2·3018
Error	20	7100·06	355·00	0·63158
Total .	29	51385·37	..	Diff.=1·67022

Two points are brought out by Table I :—

(1) The bulk of the total Variance is due to treatments and the experiment is likely to give significant results.

(2) The amount of Variance eliminated as due to Blocks is considerable and much greater than the residual amount left due to Random Error.

Apparently the design has been satisfactory.

(*b*) "*z*" *test.*—Using the table provided for "z" [Fisher 1930, 2] the significance of the whole experiment is first tested, *i.e.*, the significance of the Variance due to Treatments over Error. In this case $n_1=4$ and $n_2=20$ and the theoretical value of "z" for 100 : 1 odds=·7443, while the observed value=2·3018—0·63158=1·6702. It is therefore overwhelmingly significant.

(*c*) *Significance of Treatments.*—Having ascertained the significance of the experiment by the "z" test, comparison is made between the treatment means and the

significance tested by the S. E. of a single mean $=\sqrt{355{\cdot}0\times6}=46{\cdot}15$. A difference of three times this S. E. is taken as being significant.

TABLE II.

Treatment totals.

	Control (1)	D (2)	D+F_1 (3)	D+F_2 (4)	D+F_3 (5)	Mean	S. E.
In ¼ lbs.	1289	1703	1757	1816	1932	1699·4	46·15
Expressed as percentage of mean	75·88	100·23	103·4	106·88	113·70	100	2·72

CONCLUSION.

A Standard Error of a single mean of 2·72 per cent. was obtained and as, in manurial work such as is being carried out here, any difference due to treatment not exceeding three times this would not be worth while considering economically, it was decided to adopt this method, and in 1931 all new experiments have been laid down accordingly. It will be interesting to see how they work out.* At present they all look in splendid condition.

SUMMARY.

On a field experimental scale with paddy using Fisher's Randomised Block design cum Analysis of Variance Statistical Interpretation with five treatments, six replications and a total of thirty plots, a Standard Error of a single treatment mean of 2·72 per cent. was obtained. It is considered that for economic manurial tests this is sufficiently precise, thus permitting a great saving in land, labour and money when compared with the method previously in use, *viz.*, Student's Method modified.

REFERENCES.

Christidis (1931). *Jour. Agric. Sci.* **21**, 1, 14.
Fisher (1930, 1). Technical Communication No. 10 of the Imperial Bureau of Soil Science.
Fisher (1930, 2). Statistical Methods for Research Workers, Third edition.
Lord (1924), *Agri . Jour. of India* **19**, 1, 26.
Maskell (1929, 1). *Tropical Agric.* **5**, 12, 306.
——— (1929, 2). *Tropical Agric.* **6**, 1, 5.
——— (1929, 3). *Tropical Agric.* **6**, 2, 45.
——— (1929, 4). *Tropical Agric.* **6**, 4, 97.

* Even much lower Standard Errors have been obtained.

INHERITANCE OF CHARACTERS IN *SETARIA ITALICA* (BEAUV.).

PART II.* ANTHER COLOURS

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In *Setaria italica* the common colour of the anther on emergence is orange, with a tinge of varying degrees of brown. The dehiscence of the anthers, the emptying of the pollen, the consequent shrivelling, drying and discoloration of the anther-sacs, follow in quick succession.

The dry anthers, if gathered before they are blown off, and put into a tube present a mass of powder, blackish-brown in appearance.

Occasionally individual plants and races are met with, in which the anthers present on emergence a characteristic pale and whitish look. These whitish anthers are represented not only in Indian varieties but figure in Chinese importations also. These anthers when dry and gathered into a tube present a powdery mass, buff-yellow in colour.

These two anther colours, orange and white, are met with in both purple pigmented and green-throughout plants. They occur independently without any selective association with either colour of grain (husk) or bristle length. Not being a naked grain, their association with the colour of the seed coat (not husk) could not be gauged.†

These two anther colours behave as a simple Mendelian pair of characters and their inheritance has been pursued at the Millet Breeding Station, Coimbatore. In the year 1927 a segregation for this character was first met with. In 1928 family

* Part I. Published in this *Journal* Vol. 1, Part V, pp. 587-609.

† *Vide* "Segregation for Anther Colour in Andropogon Sorghum" by G. N. Rangaswami Ayyangar, Millet Specialist, Agricultural Research Institute, Coimbatore, in the Proceedings of the Twelfth Indian Science Congress, Calcutta 1925, published by the Asiatic Society of Bengal, 1, Park Street, Calcutta.

No. S. I. 47 was found to segregate similarly and the history of this family in subsequent generations is given below :—

Clan S. I. 47.

Generation		Family No.	Anther colour	
			Orange	White
Natural Cross (1927) F_2		S. I. 47	73	22
F_3	*Character of selection*			
	Orange	S. I. 1030	Pure	..
	,,	S. I. 1031	216	45
	,,	S. I. 1032	153	58
	,,	S. I. 1033	124	53
	White	S. I. 1034	..	Pure
F_4 (From S. I. 1032)	Orange	S. I. 1713	Pure	..
	,,	S. I. 1714	54	11
	,,	S. I. 1715	37	12
	,,	S. I. 1716	37	14
	White	S. I. 1717	..	Pure

The following other families were also noted to segregate for the same character.

Family No.	Segregation for anther colours	
	Orange	White
S. I. 555	53	14
S. I. 594	67	26
S. I. 814	75	26
S. I. 967	37	15
S. I. 968	145	68
S. I. 969	111	29
S. I. 975	87	22
S. I. 1071	49	14
Total	644	214

Artificial confirmation of this segregation was made by crossing a white-anthered mother S. I. 1261 with an orange-anthered father S. I. 1264 in crosses S. I. LVII to LXI which gave, as expected orange-anthered first generation plants.

Summary.

In *setaria italica* two fresh anther colours are met with, *viz.*, brownish-orange and white. These, when dry and seen *en masse* appear brownish-black and buff-yellow respectively. These colours form a simple Mendelian pair, with orange dominant.

SINGLE VALUE SOIL PROPERTIES OF TROPICAL SOILS

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I. Introduction.

Using 39 soils of various types, Keen and Coutts [1928] have shown that important correlations exist between single value properties of soils such as percentage of clay, air-dry moisture, moisture at 50 per cent. relative humidity, loss on ignition, sticky point, etc. In a later paper Coutts [1929] has confirmed the general findings as applied to a selection of 66 South African Soils. The main conclusions arrived at by Keen and Coutts as a result of investigation of the single values referred to above were as follows :—

1. Heavy clay soils exhibited the highest ignition losses, moisture contents and sticky points.
2. The correlation $\frac{C}{S}$ (C=clay per cent., S=H_2O content per cent. at sticky point) increased as a result of treatment with H_2O_2, thereby suggesting that the sticky point value is controlled by organic matter in addition to some property allied to the clay content.

3. Partial correlations showed that the sticky point was controlled by the colloidal matter, organic and inorganic, while the value for 'R', *i.e.*, the amount of water held by soil in an atmosphere at 50 per cent. relative humidity was controlled by the clay.

II. Scope of present enquiry.

In the first place it was intended only to test the results obtained by Keen and Coutts with a large number of soils from various surveys already in hand. The per cent. clay (C), loss on ignition (I), air-dry moisture (L), and moisture at the sticky point (S) for these soils had already been determined, and it was not proposed in the first place to attempt any special work on the subject.

III. Soils used in the investigation.

These were from three separate and distinct surveys.

(*a*) *Mandalay Canal area soils.*—These comprised 38 surface soils with their corresponding sub-soils. Practically all are calcareous paddy soils.

(*b*) *Pegu District soils.*— These comprised 119 surface soils and their corresponding sub-soils. Practically all are typically sour Lower Burma paddy soils.

(*c*) *Forest Survey soils.*—Unlike the two previous groups of soils, these are from various soil types. They comprised 53 surface soils 0-3″, with sub-soils at 1′-2′ and 3′-4′ depth. Not in every case could all relationships be examined with 53 soils and sub-soils. In certain cases only about 20 comparisons could be made. Most of these soils are unsaturated but a few are calcareous.

A special feature in Keen and Coutts' work was the use of H_2O_2 to assess the value of organic matter in its effect on certain values. In the present case it appeared possible that similar results might be obtained by comparison at various depths instead of using H_2O_2, although it was of course possible that changes in the chemical composition of the soil at various depths or horizons would obscure any such relationships.

IV. Methods used.

(*a*) *Percentage clay* (*C*).—This was determined by the method of the Agricultural Education Association [1926] with the modification that H_2O_2 was not used. It has been shown by the writer [Charlton, 1927] that H_2O_2 is not essential for maximum clay yields in tropical soils containing small amounts of organic matter.

(*b*) *Air-dry moisture* (*L*).—Determined as the loss in weight by drying 2-3 grms. soil at 100-105°C for 24 hours.

(*c*) *Loss on ignition* (*I*).—Soil from (*b*) was taken and the further loss resulting from ignition at red heat determined.

(*d*) *Moisture at the sticky point* (*S*).—The method as described by Keen and Coutts was followed but in every case the soils were made rather too wet and were then worked by hand until the warmth of the fingers reduced the soils to the desired condition. This was found to give reproducible results even in the hands of different assistants, whereas unless excess water was made at the starting point of the treatment agreement was not so good. All determinations were carried out in duplicate and where the amount of water found in the duplicates differed by two per cent. or more, the estimations were repeated until satisfactory checks were obtained. It should be noted that, in the duplicate experiments referred to, the two lots of soil and water were worked up quite independently. Also since Keen and Coutts showed that in soils at the sticky point 16 per cent. water was held as interstitial water, a round figure of 16 per cent. was deducted from the amount of water found in the soils at the sticky point. It was considered that this would give a closer relationship to the colloidal properties of the soil than the total water content at the sticky point without affecting the correlations obtained.

No other properties were examined.

It should be noted that the data are calculated on a basis of soil dried to constant weight at 100-105°C, except in the case of the determination of the clay which is on the air-dry basis. Obviously a soil dried to constant weight at 100-105°C has no air-dry moisture but the adoption of such a basis is in reality a perfectly simple matter and useful for such comparisons.

The fact that L is not identical with R, *i.e.*, the moisture retained by the soil in an atmosphere of 50 per cent. relative humidity should be remembered. Hence the comparison with Keen and Coutts' results could not be pushed too far.

V. Experimental results (preliminary).

(*a*) *Data obtained.*

These are shown in Table I in which the four single value properties C, S, L and I have been expressed as correlation co-efficients. Partial correlation co-efficients are shown in the same table. Non-significant values are shown in brackets [ ] while comparable significant differences have been shown by arrows. Comparable values not connected by arrows do not differ significantly. Certain partial correlation co-efficients in the forest soils 1′-2′ have given values greater than unity. The basis of significance in all cases has been taken at P=·05. The number of samples employed in ascertaining total correlations is shown in Table I opposite *n* which is the number of soils used. The number used for ascertaining partial correlations is not shown because this follows automatically.

TABLE I.

Correlation co-efficients for Burma soils.

Correlation	Mandalay		Pegu		Forest Soils		
	0″—6″	6″—12″	0″—6″	6″—12″	0″—3″	1′—2′	3′—4′
n=	(37)	(38)	(117)	(119)	(24)	(24)	(20)
rSI	·770	·694	·858	·849	·901	·899	·773
rSI. L	·583	·572	·662	·6[illegible]	·524	·806	·578
rSI. C	·709	·708	·723	·735	·929	1·127	·904
rSI. CL	·579	·614	·675	·686	·821	1·268	·919
n=	(37)	(38)	(117)	(119)	(24)	(24)	(20)
rSL	·764	·726	·728	·714	·873	·682	·643
rSL.I	·570	·621	[·041]	[·181]	[·288]	[—·161]	[·171]
rSL. C	·644	·615	·376	·422	·836	·686	·657
rSL. IC	·467	·472	[·160]	·244	·531	..	—·703
n=	(37)	(38)	(115)	(119)	(52)	(52)	(47)
rSC	·578	·544	·680	·628	·565	[·198]	[·141]
rSC. I	·432	.568	[·160]	[—·056]	—·71[illegible]	—1·508	—·745
rSC. L.	[·253]	[·273]	[·137]	[·075]	—·369	[—·222]	[·081]
rSC. IL	[·241]	·378	—·221	—·215	—·782	—1·628	—·876

TABLE I—*contd.*

Correlation co-efficients for Burma soils—contd.

Correlation	Mandalay		Pegu		Forest soils		
	0″—6″	6″—12″	0″—6″	6″—12″	0″—3″	1′—2′	3′—4′
n =	(38)	(38)	(117)	(119)	(25)	(24)	(20)
rCI	·427	[·208]	·844	·762	·822	·730	·648
rCI.L	[·100]	[—·064]	·432	·336	·439	6·41	·830
rCI.S	[—0·035]	[—·281]	·602	·556	·874	1·286	·858
rCI.LS	[—0·060]	[—·279]	·460	·386	·799	1·421	·963
n =	(38)	(38)	(117)	(119)	(25)	(24)	(20)
rCL	·583	·530	·871	·840	·777	·497	[·124]
rCL.I	·448	·502	·564	·614	[·116]	[—·224]	—·689
rCL.S	[·269]	[·234]	·748	·719	·705	·505	[·044]
rCL.IS	[·275]	[·231]	·578	·636	·477	..	—·851
n =	(38)	(38)	(119)	(119)	(25)	(24)	(20)
rLI	·623	·482	·835	·768	·912	·805	·737
rLI.C	·509	·448	·379	·365	·763	·746	·869
rLI.S	[·109]	[—·044]	·597	·438	·593	·599	·494
rLI.CS	[·1225]	[·023]	[·166]	[·066]	[—·067]	..	·905

(b) Comparison with results obtained by other workers.

The mathematical methods followed were the same as those employed by Keen and Coutts, these being developed by Fisher [1928].

The highest value for total correlations is ^{r}SI, and this is followed fairly closely by ^{r}SL and ^{r}LI, the two latter being approximately equal. The values obtained for ^{r}SI are close to those quoted by Coutts and by Keen and Coutts. ^{r}SI, ^{r}SL and ^{r}LI appear to be relatively unaffected by the depth from which the soil samples were drawn and hence may be independent of the organic matter. At any rate such changes in values with increased depth as were obtained are not significant. Unfortunately in the case of some of the forest soils, relatively few comparisons could be made and hence comparatively large changes in value are necessary for significance in these cases.

Of the remaining total correlations ^{r}SC appears to be affected by the depth of sampling, the correlation disappearing at even 1′-2′ in the case of forest soils while the change in value of ^{r}SC from 0″-3″ to 1′-2′ is significant. The number of samples in these cases is over fifty, so that considerable confidence can be placed in the results.

^{r}CL behaves rather similarly to ^{r}SC but the correlation disappears only at 3′-4′ in the forest soils, the fall in value from 0″-3″ to 3′-4′ being significant.

^{r}CI behaves curiously inasmuch as it gives significant values relatively unaffected by depth of sampling except in the case of the Mandalay sub-soils. This cannot be explained at present. Meanwhile it may be noted that the amount of carbon dioxide in carbonate form in soil and sub-soil respectively is approximately equal in Mandalay and Pegu groups. In Table II these data are given together with values for organic matter for the same soils. Similar data are not available for the forest soils.

TABLE II.

Chemical data regarding Mandalay and Pegu soils : (average of all soils examined per cent. on air-dry soils).

—	Mandalay soils		Pegu soils	
	0″-6″	6″-12″	0″-6″	6″-12″
Co_2 in carbonate form	1·33	1·47	0·061	0·052
Organic carbon (wet combustion) . .	1·22	0·82	1·04	0·65

Further information must therefore be sought from the partial correlations given in Table I. From these the following general conclusions may be drawn :—

rSI

1. The correlation is chiefly controlled by L and is scarcely affected by elimination of C.

2. Depth of sampling seems to be without effect on rSI.

3. The impossibly high values for rSI.C and rSI.CL for the forest soils at 1′-2′ are most probably due to changes in value with change of horizon, the unsatisfactory nature of L itself, being an estimation usually imperfectly controlled due to changes in relative humidity or to the small number of comparisons.

rSL

1. Elimination of I destroys the correlation in most cases while elimination of C has relatively little effect. Hence the correlation rSL depends chiefly on factors destroyed by ignition and depends scarcely at all on the clay content.

2. It is likely that the values for rSL.IC are relatively high because S includes the air-dry moisture L. A study of the correlation rS-L. L. would be worth making but this was not attempted.

3. The significant negative value for rSL.IC in the forest soils 3′—4′ depth may again be due to the causes mentioned under rSI No. 3 above. The fall in the value of rSL.IC from 0″—3″ to 3′—4′ is also significant.

rLI

1. The three groups of soils do not behave alike. Although elimination of clay causes a significant fall in the case of Pegu soils, the value rLI.C remains significant and in some cases is almost as high as rLI. Elimination of S destroys the correlation in Mandalay soils, is less important than elimination of C in Pegu soils and more important than C in the forest soils. In general rLI.CS is non-significant but in the case of the forest soils at 3′—4′ depth it is very highly significant.

2. In general the correlation rLI seems to depend chiefly upon the same factors which control the sticky point.

rSC

1. The total correlation disappears at 1′—2′ depth in the forest soils.

2. Although the Mandalay calcareous soils behave somewhat differently from the other soils, it is seen that elimination of I and L profoundly affect the correlation though not always to the same degree. Further, elimination of both I and L may give significant negative correlations. It is therefore likely that S and C need not necessarily be directly causally related.

^{r}CL

1. The total correlation disappears at 3′—4′ depth in the forest soils.

2. Forest soils behave quite differently from the Mandalay and Pegu soils when $^{r}CL.I$ is considered.

3. It is not advisable to draw any further conclusions from the correlations ^{r}CL except that it seems likely that change of composition of the soil with depth can transform $^{r}CL.I$ and $^{r}CL.IS$ to significant negative quantities.

^{r}CI

1. It would be expected that with the diminution in organic carbon content with increased depth of sampling, an increased total correlation ^{r}CI would be obtained. There is no sign of any such increase.

2. Partial correlations $^{r}CI.L$ and $^{r}CI.LS$ show some tendency to increase with depth but there is obviously a great difference in behaviour in the different groups of soils.

The only correlations strictly comparable with those given by Keen and Coutts are ^{r}SI, ^{r}SC and ^{r}CI. It was considered that the apparently peculiar behaviour of ^{r}SC at various depths in the sub-soils required explanation as no suggestion of such behaviour was given by Keen and Coutts. Further, the value ^{r}CI which in one case was 0·844 required explanation as Keen and Coutts obtained only 0·364 with 39 soils and Coutts later found $^{r}CI=0·751$ with 66 soils.

(c) Reasons for necessity of examination of effect of change of replaceable bases.

The soils examined were all drawn from arbitrary depths and not by horizons. It may be added that this is not a serious matter with Burma paddy soils which are usually exceedingly deep alluvial soils in which horizon changes are few, if any, except for the gradual loss of colour due to diminished organic matter with increased depth. If the conclusion arrived at by Keen and Coutts to the effect that heavy clays have high sticky points and that since ^{r}CS increases as a result of H_2O_2 treatment is valid there seems no good reason for the diminution of the correlation ^{r}SC with forest soils at 1′—2′ and 3′—4′ depth unless other important factors besides organic matter affect the correlation.

It had been noticed in laboratory work carried out for special purposes that the sticky point of sodium clays gave lower values than one would have expected from the amounts of clay present. In this connection it may be added that Smolik [1930] found that sorption of water vapour by soils in an atmosphere of 50 per cent. relative humidity was affected by the replaceable bases present. The sorption increased in the series $Na^{\cdot}$, $Ca^{\cdot\cdot}$, $Mg^{\cdot\cdot}$, $K^{\cdot}$ and $NH^{\cdot}_4$. It was therefore decided

to examine this possibility, since change of replaceable bases with change of horizons seemed a likely cause of the variations found. Another likely cause would also be the silica/sesquioxide ratio with change of soil or change of horizon but this was not examined as sufficient time was not available.

VI. Change of replaceable bases.

The soils used were taken from the Pegu Survey surface soils, 24 being taken, the only restriction being that there should be a large amount of soil available. It was decided to prepare hydrogen, calcium and sodium soils, with and without H_2O_2 pre-treatment. In addition, one lot of the soils was treated with H_2O_2 only for the sake of comparison. Magnesium, ammonium, etc., soils were not prepared.

VII. Methods used.

Hydrogen clays were produced by exhaustive treatment with $N/_{20}$ HCl until all calcium had been removed after which the soils were washed with distilled water until free from chlorides. The soils were then dried at a temperature not exceeding 45°C. Calcium soils were produced by repeated treatment with *N*. $CaCl_2$ solution, at least 15 such treatments being given. The soils were then washed free from chlorides and dried at a temperature not exceeding 45°C.

Sodium soils could not be produced successfully by comparable means as they could not be washed free from chlorides. Accordingly the fully unsaturated soils were prepared, distilled water added in the amount soil/water=$1/_2$ and $N/_{10}$ NaOH added drop by drop until the reactions were just alkaline to phenolphthalein. More $N/_{10}$ NaOH was added daily as necessary for five successive days to bring the soil to pH 8·4 approximately. The soil-water mixtures were then dried at room temperature by means of a current of air from a fan. Prepared in this way the soils were not really sodium clays but sodium-hydrogen clays since at pH 8·4 complete displacement of hydrogen is not achieved. In spite of this drawback the soils were regarded as sodium soils. One merit of the method of preparation was that loss of organic matter was avoided.

In all cases when it was desired to produce hydrogen, calcium and sodium soils after removal of organic matter, the treatment with H_2O_2 was carried out first after which the treatment for introduction of replaceable bases was identical with treatments previously described. It should be noted in this case that the use of air-dry moisture figures (L) was not possible and hence the value 'R' *i.e.*, the amount of moisture in the soil in contact with a 50 per cent. saturated atmosphere was substituted. The temperature was kept constant at 30°C and the soils were exposed in very thin layers at the bottom of special wide, shallow weighing bottles until no

further change in weight was recorded. This was usually after about five days' exposure.

VIII. Experimental results.

(*a*) *Data obtained.*

These are given in Tables III and IV. In Table III the actual values for S, I and R are given while in Table IV the corresponding correlation coefficients are shown. In this connection it may be noted that as the 24 soils used were merely a rough selection covering ranges from very light to heavy soils, comparison of means is out of the question, there being no tendency to group round a mean. On the other hand it is possible to compare the differences in S, I and R produced in each of the 24 soils as a consequence of treatment received so that the result of each treatment can be assessed.

TABLE III.

Experimental data for S, I and R.

Soil No.	Soils as sampled					H_2O_2 treated only			Calcium			Hydrogen		
	Clay (air-dry) per cent.	S	I	I (air-dry) per cent.	R	S	I	R	S	I	R	S	I	R
3 . .	18·5	24·3	6·43	5·09	3·528	..	..	..	29·2	6·86	2·422	32·7	7·56	3·591
4 . .	9·0	14·7	4·34	3·29	2·283	11·7	3·33	1·881	14·6	4·39	1·693	16·9	5·01	1·923
6 . .	19·0	22·2	6·15	5·76	3·964	..	..	..	29·9	7·0	3·596	20·8	7·34	3·098
15 . .	19·0	29·8	6·52	4·88	3·985	24·7	6·49	3·237	31·8	7·32	3·152	31·6	7·47	3·050
16 . .	18·5	30·5	6·46	5·55	4·285	27·5	6·42	3·469	32·6	7·54	3·472	31·7	7·47	3·453
17 . .	8·7	24·7	5·34	2·57	2·701	19·9	5·17	2·615	25·0	6·45	2·590	26·5	6·30	2·640
18 . .	2·5	3·8	2·33	1·36	0·9452	5·0	1·69	0·620	7·4	2·50	0·627	6·5	2·40	0·912
19 . .	8·5	15·0	3·91	2·76	1·990	12·5	3·54	1·593	17·3	4·57	1·827	17·5	4·03	1·901
20 . .	6·4	12·1	3·46	1·72	1·632	11·1	3·50	1·175	13·8	4·14	1·684	14·9	3·94	1·869
21 . .	7·1	20·2	5·02	2·35	2·693	17·9	4·39	1·758	20·1	5·91	2·314	24·3	5·27	2·954
22 . .	16·5	19·6	5·53	4·06	2·641	17·1	4·68	1·763	19·0	6·17	2·617	22·2	6·03	3·026
23 . .	33·5	35·1	9·14	7·03	5·148	37·0	8·73	4·464	42·9	10·28	4·941	43·9	9·41	5·916
24 . .	12·6	18·8	4·90	2·12	2·493	15·8	4·05	1·417	22·7	5·33	2·116	24·2	5·46	2·152
25 . .	19·2	29·2	6·74	3·52	3·840	25·9	6·50	3·073	31·6	7·61	3·599	32·9	7·29	3·458
26 . .	14·5	17·2	4·57	3·63	2·488	14·3	4·40	1·695	18·7	5·16	1·966	20·4	5·20	2·016
27 . .	15·0	24·7	5·65	4·54	3·310	20·8	5·42	2·498	26·3	6·98	2·813	30·1	6·60	2·838
28 . .	12·8	24·4	5·34	2·63	2·799	18·3	5·07	2·097	25·0	6·29	2·505	26·4	6·18	2·524
29 . .	14·9	28·6	6·61	3·10	3·468	26·6	6·42	2·830	33·6	7·61	3·266	33·9	7·27	3·199
30 . .	10·5	19·4	4·57	3·69	2·707	17·6	4·06	1·705	24·1	5·56	2·260	23·8	5·40	2·112
31 . .	21·0	18·2	5·36	4·88	3·891	18·8	5·29	2·800	21·9	6·54	3·030	23·9	6·39	3·123
32 . .	23·0	28·9	7·34	4·60	4·660	26·3	7·02	4·321	33·4	8·59	4·118	31·3	8·21	4·447
33 . .	16·8	26·5	5·99	3·16	3·306	20·8	5·24	2·984	29·3	6·70	2·944	29·0	6·43	2·917
34 . .	15·7	35·6	7·63	4·25	4·613	33·0	7·40	4·196	37·8	8·54	4·365	41·2	8·29	4·592
35 . .	13·5	21·8	5·06	3·08	2·953	19·9	4·75	2·552	22·7	5·98	2·717	26·4	5·87	2·772

TABLE III—*contd.*

Experimental data for S, I and R—contd.

Soil No.	Sodium			H_2O_2 treated Ca·· clay			H_2O_2 treated H· clay			H_2O_2 treated Na· clay		
	S	I	R	S	I	R	S	I	R	S	I	R
3 . .	22·2	6·56	3·138	..	..	..	..	..	..	19·3	5·66	3·054
4 . .	9·6	3·79	1·778	12·3	3·51	1·887	14·3	3·25	1·799	11·3	3·25	1·851
6 . .	21·8	6·23	3·158	..	..	..	..	..	..	20·1	5·45	3·368
15 . .	23·5	6·67	3·306	25·9	6·00	3·296	29·4	6·05	3·288	22·0	5·65	3·044
16 . .	25·5	6·76	3·473	27·6	6·01	3·575	29·4	6·06	3·624	24·3	5·45	3·309
17 . .	18·7	5·75	2·723	21·2	5·22	2·742	22·7	4·83	2·698	15·0	4·30	2·580
18 . .	6·5	1·92	0·738	4·7	1·74	·594	5·5	1·58	0·617	7·0	1·53	·719
19 . .	11·2	3·88	1·639	13·1	3·61	1·597	14·2	3·38	1·643	8·1	3·24	1·508
20 . .	10·9	3·64	1·768	12·2	3·40	1·864	11·6	3·24	1·602	9·7	2·85	1·414
21 . .	15·7	5·01	2·078	18·4	4·16	2·483	18·7	4·18	2·371	12·2	3·65	2·110
22 . .	15·5	5·70	2·537	16·6	4·74	3·074	17·7	4·63	2·536	12·8	4·09	2·386
23 . .	36·6	9·26	4·537	37·7	8·79	5·531	39·1	8·70	5·071	29·2	7·39	4·643
24 . .	13·5	4·54	1·891	18·6	4·35	1·942	18·9	4·10	1·914	11·1	3·73	2·165
25 . .	25·7	6·76	3·215	25·1	6·31	3·981	30·5	6·31	3·477	21·3	5·56	3·234
26 . .	12·4	4·77	1·850	15·6	4·36	2·238	17·7	4·47	2·017	10·3	4·06	2·046
27 . .	22·4	6·13	2·604	23·7	5·51	3·081	25·9	5·69	2·925	18·2	4·96	2·696
28 . .	18·9	5·51	2·272	19·9	5·19	2·842	21·5	5·40	2·575	15·1	4·31	2·349
29 . .	24·8	6·81	2·874	26·1	6·54	3·461	27·7	6·85	3·263	21·3	5·32	3·066
30 . .	14·1	4·64	1·877	17·7	4·25	2·247	20·1	4·29	1·955	13·6	3·60	1·831
31 . .	15·0	5·37	3·074	19·1	5·24	3·129	22·7	5·26	3·000	15·4	4·69	3·359
32 . .	25·7	7·64	3·921	27·7	7·06	4·848	32·1	6·94	4·427	27·9	5·87	4·124
33 . .	18·8	5·99	2·898	22·7	5·30	3·199	24·5	4·94	3·003	17·1	4·52	2·787
34 . .	32·0	7·83	4·150	36·0	7·54	4·512	36·4	7·17	4·467	31·2	6·47	3·988
35 . .	16·5	5·14	2·346	20·8	5·06	2·860	22·9	4·69	2·730	17·1	4·48	2·659

TABLE IV.

Correlation co-efficients for Burma paddy soils as modified by change of replaceable bases.

Correlation	Untreated (24)	H_2O_2 treated only (22)
r_{SI}	·939	·977
$r_{SI.R}$	·615	·829
$r_{SI.C}$	·906	·940
$r_{SI.CR}$	·756	·838
r_{SR}	·901	·925
$r_{SR.I}$	[·110]	[·054]
$r_{SR.C}$	·808	·783
$r_{SR.IC}$	[·406]	[·072]
r_{SC}	·725	·803
$r_{SC.I}$	—·535	[—·312]
$r_{SC.R}$	[—·354]	[·220]
$r_{SC.IR}$	—·629	[—·316]
r_{CI}	·869	·857
$r_{CI.R}$	[·213]	·455
$r_{CI.S}$	·794	·570
$r_{CI.RS}$	·584	·500
r_{CR}	·884	·816
$r_{CR.I}$	[·385]	[·046]
$r_{CR.S}$	·772	[·324]
$r_{CR.IS}$	·530	[·065]
r_{RI}	·947	·943
$r_{RI.C}$	·770	·818
$r_{RI.S}$	·673	·486
$r_{RI.CS}$	[·155]	[·387]

TABLE IV—*contd.*

Correlation co-efficients for Burma paddy soils as modified by change of replaceable bases—contd.

Correlation	$Ca^{\cdot\cdot}$ treated only (24)	$H^{\cdot}$ treated only (24)	$Na^{\cdot}$ treated only (24)
rSI	·964	·959	·968
rSI. R	·693	·754	·722
rSI. C	·915	·911	·921
rSI. CR	·739	·807	·744
rSR	·931	·904	·932
rSR. I	[—·135]	[·141]	[·025]
rSR. C	·808	·747	·818
rSR. IC	[—·163]	[·307]	[·148]
rSC	·786	·767	·783
rSC. I	[—·351]	[—·361]	[—·270]
rSC. R	[·043]	[—·052]	[—·161]
rSC. IR	[—·370]	—·441	[—·303]
rCI	·864	·855	·846
rCI. R	[·406]	[·298]	[·066]
rCI. S	·647	·657	·564
rCI. RS	·522	·516	[·267]
rCR	·835	·861	·871
rCR. I	[—·058]	[·351]	[·393]
rCR. S	·458	·611	·626
rCR. IS	[—·111]	·436	[·416]
rRI	·974	·927	·961
rRI. C	·912	·723	·855
rRI. S	·789	·495	·646
rRI. CS	·727	[·157]	·455

TABLE IV—*contd.*

Correlation co-efficients for Burma paddy soils as modified by change of replaceable bases—contd.

Correlation	(H_2O_2 treated) —$Ca^{\cdot\cdot}$ (22)	(H_2O_2 treated) —$H^{\cdot}$ (22)	(H_2O_2 treated)—$Na^{\cdot}$ (24)
r_{SI}	·979	·972	·934
$r_{SI.R}$	·830	·587	·425
$r_{SI.C}$	·964	·910	·859
$r_{SI.CR}$	·868	·615	·512
r_{SR}	·935	·966	·923
$r_{SR.I}$	[—·248]	·454	[·225]
$r_{SR.C}$	·844	·891	·843
$r_{SR.IC}$	[—·014]	·509	·430
r_{SC}	·774	·826	·759
$r_{SC.I}$	—·563	[—·067]	[—·357]
$r_{SC.R}$	[—·290]	—·137]	—·412
$r_{SC.IR}$	—·521	[—·258]	—·500
r_{CI}	·852	·858	·878
$r_{CI.R}$	[·001]	[·122]	[·091]
$r_{CI.S}$	·730	·415	·728
$r_{CI.RS}$	·454	[·252]	[·327]
r_{CR}	·880	·873	·898
$r_{CR.I}$	[·421]	[·334]	·403
$r_{CR.S}$	·696	·515	·789
$r_{CR.IS}$	[·353]	·410	·530
r_{RI}	·968	·965	·966
$r_{RI.C}$	·877	·862	·843
$r_{RI.S}$	·727	·427	·757
$r_{RI.CS}$	·446	[·273]	·433

TABLE IV—*concld.*

Correlation co-efficients for Burma paddy soils as modified by change of replaceable bases—concld.

Correlation	Keen and Coutts' data (39 soils)		Coutts' data for 66 South African soils
	Untreated	H_2O_2 treated	
rSI	·865	·879	·928
rSI. R	·841	·873	·755
rSI. C	·844	·781	·837
rSI. CR	·847	·851	·722
rSR	·503	·584	·859
rSR. I	·362	·556	·434
rSR. C	·366	[·148]	·691
rSR. IC	·382	·553	[·404
rSC	·371	·675	·743
rSC. I	[·120]	[·260]	[·188]
rSC. R	+[·016]	·438	·326
rSC. IR	[—·180]	[—·257]	[·067]
rCI	·364	·662	·751
rCI. R	[·133]	—·615	·378
rCI. S	[·092]	[·195]	·247
rCI. RS	[·222]	·530	[·213]
rCR	·719	·760	·733
rCR. I	·673	·729	·300
rCR. S	·663	·611	·276
rCR. IS	·680	·729	·247
rRI	·388	·386	·828
rRI. C	[·195]	—[·240]	·618
rRI. S	[—·109]	—·329	[·162]
rR1. CS	[—·229]	—·577	[·101]

(b) Effect of change of replaceable bases on S, I and R.

In Table V the effect of the change of replaceable bases on the sticky point moisture has been shown. All differences produced are significant except that between the untreated soil and the H· soil which has received H_2O_2 treatment. It must be noted that in Tables V, VI and VII although non-significance is shown by double ended arrows under mean value for the sake of convenience, the significance or non-significance does not apply to the means but to the differences produced by treatment. It is obvious that the moisture at the sticky point is profoundly affected by change of replaceable bases, the order for S being H· > Ca·· > Na· whether organic matter is present or not. The removal of organic matter in general reduces the S value by 3·2 per cent. but although this is a considerable effect it is much smaller than the differences between H· and Na· soils which may amount to 7·7 per cent. in the presence of organic matter and 5·8 per cent. after H_2O_2 treatment.

The fact that in podsol soil in which clay accumulates in the B horizon the sticky point moisture of the 'B' horizon was not increased over that of the 'A' horizon, has been noted by Scott-Blair [1931]. This may possibly be due to the presence of different replaceable bases in A and B horizons.

TABLE V.

Change of sticky point with change of replaceable bases and H_2O_2 treatment.

Soil	Mean value of sticky point	Mean change
H·	26·75	1·304
Ca··	25·45	2·186
H_2O_2 treated —H·	→22·89	0·214
Untreated —H·	→22·72	1·641
H_2O_2 treated —Ca··	21·03	0·918
H_2O_2 only	20·11	1·318
Na·	19·06	1·954
H_2O_2 treated —Na·	17·11	

TABLE VI.

Change of loss on ignition with change of replaceable bases and H_2O_2 treatment.

Soil	Mean value of loss on ignition	Mean change
Ca··	→6·418	0·133
H·	→6·284	0·605
Na·	→5·680	0·080
Untreated	→5·600	0·360
H_2O_2 treated —Ca··	→5·177	0·015
H_2O_2 only	→5·162	0·070
H_2O_2 treated —H·	→5·091	0·593
H_2O_2 treated —Na·	4·587	

TABLE VII.

Change of 'R' with change of replaceable bases and H_2O_2 treatment.

Soil	Mean value of 'R'	Mean change
Untreated	3·180	0·175
H_2O_2—Ca··	→2·954	0·053
H·	→2·938	0·162
Ca··	→2·776	0·018
H_2O_2—H·	→2·773	0·138
H_2O_2—Na·	→2·683	0·022
Na·	→2·660	0·128
H_2O_2 only	2·488	

Table VI shows the changes produced in I by changes of replaceable bases and H_2O_2 treatment. Absence of arrows indicates significant differences produced.

In this case the differences are not so marked as in the case of the values of S but are well marked, none the less. The values of I are in the order $Ca^{\cdot\cdot}=H^{\cdot} > Na^{\cdot}$ whether treated with H_2O_2 or not. Removal of organic matter by H_2O_2 reduced I on the average by one per cent. which is greater than the change produced by different replaceable bases. This is as would be expected.

The results obtained by change of replaceable bases and H_2O_2 treatment on the value of R are given in Table VII where again absence of arrows indicates significant differences. The values are not so satisfactory as in the cases of S and I. The value of R for untreated soil remains definitely higher than the values after any treatment whereas in the case of S and I certain treatments gave values both higher and lower than those of untreated soils. This is unsatisfactory but although it would appear likely that the treatments which the soils were subjected to are responsible, it is possible that some replaceable base not tried in these experiments would have exhibited higher values for 'R' than was obtained for the untreated soils. In this connection it may be pointed out that Smolik found R to increase in the series $Na^{\cdot}$, $Ca^{\cdot\cdot}$, $Mg^{\cdot\cdot}$, $K^{\cdot}$ and $NH_4^{\cdot}$ so that removal of replaceable $Mg^{\cdot\cdot}$, $K^{\cdot}$, and $NH_4^{\cdot}$ may be responsible for the low value of R obtained. In the series obtained, the values are in the order $H^{\cdot} > Ca^{\cdot\cdot} > Na^{\cdot}$ without H_2O_2 treatment and $Ca^{\cdot\cdot} > H^{\cdot} = Na^{\cdot}$ after H_2O_2 treatment. The mean reduction in the value of R as a result of H_2O_2 treatment is 0·16 per cent. but this is subject to many exceptions and is not to be relied upon. Comparison between the relative effects of H_2O_2 treatment and change of replaceable base is not possible from the data obtained.

It may be stated that whereas S is chiefly controlled by the replaceable bases, followed by the organic matter, I is controlled chiefly by the organic matter and to a less extent by the replaceable bases while the facts concerning R are uncertain. It should be remembered that all the soils quoted are typical tropical arable soils usually containing only about one per cent. organic carbon. In temperate climates it is possible that organic matter may be the chief controlling factor for S, I and R because of its greater amount in such soils.

It will be noted that in general, the effect of substitution of various replaceable bases such as $H^{\cdot}$, $Ca^{\cdot\cdot}$ and $Na^{\cdot}$ tends to affect S, I and R in the same way or direction, *i.e.*, increase in the value of S is usually accompanied by increase in the value of I and R but this is not invariable. Thus the highest sticky points are found in $H^{\cdot}$ soils but the loss on ignition of $H^{\cdot}$ soils is greater than with $Ca^{\cdot\cdot}$ soils. Further, although in soils not treated with H_2O_2, the values of R are $H^{\cdot} > Ca^{\cdot\cdot} > Na^{\cdot}$, in the H_2O_2 treated soil the order is $Ca^{\cdot\cdot} > H^{\cdot} = Na^{\cdot}$. Although $Mg^{\cdot\cdot}$, $K^{\cdot}$ and $NH_4^{\cdot}$ soils were not prepared, it is obvious that the possibility of high values of S being produced with low values of I or R by certain replaceable bases is not excluded and may lead to negative correlations.

(c) *Effect on correlation coefficients.*

These are shown in Table IV. The values obtained by Keen and Coutts and by Coutts have also been entered for ease of comparison. In this connection it may be noted that the value reported for rSC by Keen and Coutts [1928] for untreated soils is 0·371 and not 0·317 as shown in the original paper*. The correlations are as calculated by the writer and do not in all cases agree with those given by Keen and Coutts.

rSI

The substitution of different replaceable bases has comparatively little effect and the values both for total and partial correlations agree excellently with the values obtained by Coutts for 66 South African soils. The value for rSI.CR for H_2O_2 treated $Ca^{\cdot\cdot}$ soil is significantly greater than the value for H_2O_2 treated $Na^{\cdot}$ soil. Partial correlations show that elimination of R causes a small significant reduction but elimination of clay is without significant effect on the correlation.

rSR

The resemblance to Coutts' results for 66 South African soils is well marked. In the majority of cases rSR.C is significantly greater than rSR.I and hence while elimination of clay is comparatively without effect, elimination of I in most cases destroys or greatly reduces the correlation. It is only in the case of $H^{\cdot}$ and $Na^{\cdot}$ soils after treatment with H_2O_2 that the correlation rSR.IC is significant. This is partly due to the small number of soils used in the tests. There is little doubt that certain replaceable bases may cause an inverse relationship in S and R.

rSC

Keen and Coutts found that the correlation increased very considerably as a result of H_2O_2 treatment. Their figure rSC=0·371 for untreated soil is remarkably low and Coutts' figure for 66 South African soils agrees much better with the values for rSC for Burma soils. Elimination of I destroys the correlation or transforms it into a significant negative value. The treatment with H_2O_2 is not responsible for this negative value which occurs both in untreated and H_2O_2 treated soils. Elimination of R also gives negative values though these are rarely significant. Organic matter plays a more important part in rSC.R than in rSC.I. A remarkably high significant negative correlation for rSC.IR is obtained for untreated soils both with and without H_2O_2 treatment. Keen and Coutts likewise obtain a non-significant negative correlation for rSC.IR both with and without H_2O_2 treatment. Coutts' figures do not agree well with any of the rSC figures for Burma soils. It is clear

* Private communication from J. R. H. Coutts.

that an inverse relationship may be expected between S and C and this could easily arise if in the case of heavy clay soils these were chiefly combined with $Na^{\cdot}$ while the lighter soils would be chiefly $H^{\cdot}$ soils. The possible part played by replaceable bases other than $H^{\cdot}$, $Ca^{\cdot\cdot}$ and $Na^{\cdot}$ cannot of course be stated for lack of experimental data.

rCI

Again the values obtained by Coutts for rCI agree better with the values found for Burma soils than the values obtained by Keen and Coutts. Partial correlations do not agree well with Coutts' or Keen and Coutts' results however, as in Burma soils elimination of R destroys the correlation in nearly every case while elimination of S has little effect. It seems clear that the correlation rCI depends to a great extent upon the replaceable bases present in spite of the fact that the small amounts of organic matter present in these soils individually alter the loss on ignition figure more than change of replaceable base. Whereas Keen and Coutts obtained an increased correlation rCI after H_2O_2 treatment, in the case of the 24 Burma paddy soils no increase was obtained. Also in the case of the values for rCI quoted in Table I, in every case without exception the value rCI diminished with increased depth although the diminution was in no case significant.

rCR

The correlations do not agree well with those obtained by Keen and Coutts and by Coutts. In the majority of cases rCR.I is non-significant in Burma soils whereas rCR.S is generally significant. The conclusion is that rCR depends largely upon the presence of organic matter but also to a large extent upon the replaceable bases.

rRI

In this case again the values obtained for Burma soils agree better with Coutts' results than with those of Keen and Coutts. The close agreement between the values obtained by Coutts and the writer for rRI and the much lower value obtained by Keen and Coutts suggests that this particular correlation is considerably affected by soil type. The very high total correlation seems independent of the presence of organic matter but partial correlations show that R and I are not highly correlated except as $Ca^{\cdot\cdot}$ soils for which rRI. CS is remarkably high. It follows that with certain replaceable bases rRI may be high but as the relationship depends upon the clay and the factors controlling the sticky point as well, R and I are not usually highly casually related.

The fact that Keen and Coutts found a significant negative value for rRI. CS for H_2O_2 treated soils probably indicates that under special conditions of replace-

able bases present, inverse effects on R and I may be produced, thereby giving negative correlations. The values for the correlations in Table IV in respect of 39 soils reported by Keen and Coutts are as worked out by the writer. As has already been mentioned Coutts has pointed out a printing error in the original publication but the values found for ^{r}SC. I, ^{r}RI. S and ^{r}IR. S (H_2O_2—treated) by the writer differ from those quoted by Keen and Coutts. Coutts* has now accepted all the values as worked out by the writer except for ^{r}CR. S for untreated soils and hence the conclusions drawn by Keen and Coutts require some re-examination especially as regards the relationships between R and I.

From the values	^{r}SI.C	0·844
	^{r}RC.I	0·673
	^{r}RI.C	0·195
	^{r}SC.I	0·120

i.e., the values given in Table IV, Keen and Coutts drew the important conclusions that the sticky point is chiefly controlled by the material in the soil lost on ignition while R is controlled largely by the clay content. It would appear that although these conclusions apply to the 39 soils examined, they do not apply to soils in general as the 66 soils examined by Coutts and the Burma paddy soils give quite a different result.

IX. Conclusions and summary.

1. The existence of a high correlation between the sticky point moisture and loss on ignition is confirmed. The values found for ^{r}SI and partial correlations approximate to those found by Coutts for 66 South African soils. Among the soil properties examined it seems unlikely that any correlation except ^{r}SI may be regarded as relatively free from the influence of replaceable bases or other factors. Even in the case of ^{r}SI certain factors have an influence on the correlation.

2. Although high correlations were found for ^{r}SC and ^{r}RI, such relationships do not indicate a fundamental direct relationship in all cases when examined by partial correlations. There is little doubt, for instance, that clay per cent. and sticky point moisture may be inversely related in certain soils. It is believed that the variability of the relationship between clay per cent. and sticky point moisture is largely controlled by the replaceable bases although other factors may be important also.

3. The increase in the value of ^{r}SC as a result of treatment by H_2O_2 was found to be small whereas Keen and Coutts found a large increase. This may be expected since the Burma soils described have little organic matter whereas those worked with by Keen and Coutts most probably contained larger amounts. In

*Further private communication from J. R. H. Coutts.

normal arable tropical soils it follows that the sticky point moisture is largely controlled by the inorganic colloids. Experimentally it was found that the nature of the replaceable bases exercised a very large effect on the value of the sticky point moisture but as the ignition loss is somewhat similarly affected by change of replaceable base, the correlation SI remains high.

4. No evidence was found of an increased value for the correlation rCI as a result of H_2O_2 treatment or with increased depth and hence diminished organic matter.

5. It seems likely that fluctuations in the values of S, I and R are so largely under the control of the replaceable bases that it is unsafe to regard S as being controlled by amount of colloids alone, the nature of these colloids being equally important. Similarly the conclusion that R is controlled by the clay is an unsafe generalisation. Although Keen and Coutts found rCR.IS = 0·680 and 0·729 for untreated and H_2O_2-treated soils respectively, Coutts later found with South African soils the value rCR. IS = ·247 which is very significantly less than the former figures. In the case of Burma soils the value of rCR. IS was found to be ·530 for untreated soils, practically *nil* for soils treated with H_2O_2 only, while barely significant values were obtained for soils with only one replaceable base present in most cases.

6. With only one replaceable base present, the total correlations rSI and rRI approximate to perfect correlations. This indicates that even if the silica/sesquioxide ratio of soils affects the values of S, I and R, it probably has smaller effect than change of replaceable base. On the other hand the soils worked with were from an area where the silica/sesquioxide ratio may be expected to be reasonably constant so that soils from different types may be expected to give lower correlations even with only one replaceable base present.

7. Since Coutts has accepted the values obtained by the writer for the 39 soils originally worked with by Keen and Coutts except for the value rCR. S, certain conclusions drawn by Keen and Coutts require re-examination, especially the relationships between S and C, and R and I which may apparently be negatively correlated in certain soils.

8. From the values of rSI. C, rRC. I, rRI. C and rSC. I Keen and Coutts drew the conclusions that the sticky point is chiefly controlled by the material in the soil driven off by ignition while the value R is controlled more by the clay content. The values obtained by the writer and also by Coutts for 66 South African soils do not support this conclusion. Obviously the values for these partial correlations obtained by Keen and Coutts cannot be expected with all soils and other factors beyond those considered by Keen and Coutts must be taken into consideration.

The thanks of the writer are due to several of his staff for much laborious mathematical work and especially to Mr. V. V. Subramaniam, M.A., who has now left his staff to take up an appointment in the Income-tax Department, Rangoon.

REFERENCES.

Agricultural Education Association (1926) . .	*Jour. Agric. Sci.* **16**, 123.
Charlton (1927)	*Agric. Res. Inst., Pusa Bulletin* No. 172.
Coutts (1929)	*Jour. Agric. Sci.* **19**, 325.
Fisher (1928)	Statistical Methods for Research Workers—2nd Edition, Oliver and Boyd, Edinburgh.
Keen and Coutts (1928)	*Jour. Agric. Sci.* **18**, 740.
Scott-Blair (1931)	*Soil Sci.* **31**, 4, 291.
Smolik (1930)	*Bull. Zecho-Slovak. Acad. Agric.* 1930, 10.

SELECTED ARTICLE

INDIAN BARLEYS

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The Indian Provincial Governments have recently set on foot comprehensive schemes for improving the quality of barley grown in their respective districts. They have requested the assistance of the Institute of Brewing in the valuation of the barleys grown and several series of samples have already been examined and reported upon by the Valuation Sub-Committee of the Barley Research Committee. The most recent and comprehensive series of barleys was received from the Cerealist to Government, Punjab, Lyallpur Agricultural College, and these represent such an advance on the types of Indian barley hitherto known in this country that the Valuation Sub-Committee have been able to report very favourably upon them. The valuers were greatly attracted by some of the two-rowed barleys. The malts from certain of them compared closely with the best English malt. Their freedom from damage was particularly remarked upon. Others approached mid-European barleys though they differed somewhat in appearance from these. It should be borne in mind that the samples examined did not represent commercial barleys, but were from seed experimentally grown.

The majority of the six-rowed barleys were small in size, comparable with Benghazi and Egyptian, and much smaller than Californian, Tunis, or Hama barleys. The brewers' extracts shown in the detailed report are exceedingly high, when the size of the barley is taken into consideration and this, coupled with the low figures for malting loss, indicates a potentially very valuable malting material. In fact, many of the results calculated back to the brewers' extract obtainable from the quarter of raw barley are among the highest ever recorded from the quarter of any barley, British or imported. In colour and general appearance most of the barleys were unexceptionable.

The barleys were malted in stocking during March, 1930, in favourable weather. They were steeped at 57° F. and were floored for nine days at temperatures varying from 60° F. to 64° F. during the last three days and cured at 180° F. The malting called for no remark, the germination was excellent and regular, and, in most cases, the modification proceeded very well indeed, as the extracts and malting losses show.

Although these barleys differed considerably in type and variety, it is interesting to note how closely the extracts obtained agreed with those calculated

from a formula shown by Bishop to apply to English barleys. In calculating the following predicted extracts, 3·7 lb. was subtracted from the extract calculated by the formula given by Bishop for Plumage-Archer barley (*The Journal of the Institute of Brewing*, 1930, 422), the formula used becoming :—

$$E=106{\cdot}4-11{\cdot}2N+0{\cdot}18G.$$

In the list below the determined and predicted extracts and differences are given :—

Extracts in Brewers' lb. per 336 dry *Malt*—6-Rowed Barley.

Variety	Determined Extract	Predicted Extract	Difference	Variety	Determined Extract	Predicted Extract	Difference
Multan	99·9	99·2	+0·7	155 B	91·5	92·0	—0·5
Mianwali	99·5	98·4	+1·1	Naushera, common	95·4	97·1	—1·7
S. 18	99·2	98·1	+1·1	4-0303	93·8	96·3	—2·5
7-0315	95·0	95·0	0·0	Cross N/R	98·7	98·4	+0·3
S. 65	92·8	94·1	—1·3	Ludhiana	98·2	97·7	+0·5
Naushera 4-rowed	96·6	96·5	+0·1	S. 35/1	97·6	97·3	+0·3
S. 83	100·1	98·0	+2·1	152	99·2	97·5	+1·7
Lyallpur E	100·0	97·8	+2·2	155, 1926-27	98·7	98·0	+0·7
Simla	98·0	98·4	—0·4	143	99·3	98·1	+1·2
Baluchistan	96·1	96·4	—0·3	Lyallpur B. S.	97·2	97·3	—0·1
S. 66	92·9	96·5	—3·6	Hoshiarpur	96·4	98·0	—1·6
S. 7/1	99·2	98·4	+0·8	S. 60	97·3	97·6	—0·3
Gujarkhan	97·2	98·0	—0·8	124	97·3	98·7	—1·4
Gujrat	95·6	96·2	—0·6	Rewari	100·9	99·7	+1·2
5-0577	87·4	88·7	—1·3	9-2028	98·3	96·1	+2·2
122	95·4	95·7	—0·3	6-099	96·6	96·3	+0·3
8-0889	91·1	92·7	—1·6	Special No. 8	96·8	96·6	+0·2
Cape B. S.	96·0	97·6	—1·6	155A	93·2	91·3	+1·9
10-3651	96·4	95·5	+0·9				
S. 185	97·7	98·1	—0·4				

Standard Error = ±1·4 lb.

2-Rowed Barley.

Extracts predicted by Plumage-Archer Formula, E=110·1 −11·2 N+0·18G.

Variety	Determined Extract	Predicted Extract	Difference
1-0624	97·3	98·3	—1·0
2-0297	99·2	99·2	0·0
Special 2-rowed	99·3	100·5	—1·2
3-0121	100·8	99·0	+1·8
Goldthorpe	98·3	97·1	+1·2
Carter's Prolific	96·5	96·7	—0·2

Indian Barleys, 1929, and Malts.

Name	Barley			Malt					
	Moisture per cent.	1,000 corn weight grams	Nitrogen per cent. on dry barley	Moisture per cent.	Extract lb. per quarter on dry	Colour	Diastatic power °Lintner	Cold water ext., per cent.	Permy. sol. N. per cent. on dry
			6-rowed Barleys.						
Cross N/R	10·3	39·9	1·360	1·78	98·7	6·7	37·0	22·8	0·457
S 185	10·5	35·3	1·317	1·98	97·7	9·0	27·0	21·9	..
Rewari	10·6	43·2	1·305	1·96	100·9	6·0	32·0	20·2	..
155-B	10·2	44·7	2·075	2·10	91·5	7·0	48·0	19·7	0·600
152	10·3	33·8	1·345	1·80	99·2	6·2	28·5	20·7	..
152, 1926-27	10·6	40·7	1·411	2·06	98·7	7·0	28·0	19·6	..
7-0315	10·5	33·4	1·572	1·78	95·0	3·5	17·5	17·3	0·400
122	10·5	35·4	1·522	1·90	95·4	4·3	26·5	20·0	..
Lyallpur, E	10·4	36·8	1·363	2·22	100·0	6·0	36·0	20·9	..
S 83	10·3	34·7	1·313	2·06	100·1	7·0	30·5	21·3	0·454
S 60	10·6	29·2	1·256	1·88	97·3	4·0	30·5	17·2	..
155-A.	10·3	40·6	2·030	2·06	93·2	6·2	59·0	20·3	..

Indian Barleys, 1929, and Malts—contd.

Name	Barley			Malt					
	Moisture per cent.	1,000 corn weight grams	Nitrogen per cent. on dry barley	Moisture per cent.	Extract lb. per quarter on dry	Colour	Diastatic power °Lintner	Cold water ext., per cent.	Permy. sol. N. per cent. on dry
			6-rowed Barleys—contd.						
143 . . .	10·3	36·5	1·328	2·26	99·3	4·3	27·5	17·3	..
S 7/1 . .	10·2	36·7	1·305	2·12	99·2	5·0	32·0	19·5	..
Mianwali . .	10·6	34·3	1·274	1·86	99·5	4·5	26·0	18·7	..
Simla . .	10·5	28·3	1·175	1·76	98·0	7·0	26·5	22·7	0·395
Gujrat . .	10·2	26·3	1·333	1·94	95·6	6·0	27·5	19·1	..
4-0303 . .	10·0	27·4	1·347	1·70	93·8	4·8	33·0	22·0	..
Naushera, 4-rowed	10·4	30·5	1·367	1·78	96·6	4·8	23·0	18·8	0·400
Ludhiana . .	10·2	29·3	1·253	1·76	98·2	5·3	27·5	19·8	..
Hoshiarpur .	10·4	30·0	1·243	2·08	96·4	5·2	23·5	19·8	..
Gujarkhan .	10·7	29·2	1·225	1·72	97·2	5·5	26·0	19·4	..
Lyallpur, B. S. .	10·3	30·8	1·313	2·02	97·2	4·5	33·5	19·9	0·403
Naushera, Common	10·4	30·3	1·320	1·94	95·4	5·0	21·5	18·1	0·345
6-099 . .	10·2	27·5	1·349	1·98	96·6	4·2	31·5	19·5	0·410
Cape, B. S. .	10·5	28·3	1·242	1·80	96·0	4·5	26·5	18·5	0·360
S 35/1 . .	10·2	30·3	1·309	2·10	97·6	5·0	27·5	19·2	..
S 65 . . .	10·4	29·7	1·572	2·08	92·8	6·0	39·5	20·9	0·531
S 66 . . .	10·5	32·2	1·406	2·16	92·9	7·5	30·5	24·3	..

Indian Barleys, 1929, and Malts—contd.

Name	Barley: Moisture per cent.	Barley: 1,000 corn weight grams	Barley: Nitrogen per cent. on dry barley	Malt: Moisture per cent.	Malt: Extract lb. per quarter on dry	Malt: Colour	Malt: Diastatic power °Lintner	Malt: Cold water ext., per cent.	Malt: Permy. sol. N. per cent. on dry.
			6-rowed Barleys—concld.						
5-0577 . .	10·5	29·2	2·050	1·96	87·4	3·7	38·0	20·0	0·555
Baluchistan .	10·2	29·8	1·375	1·90	96·1	5·2	27·0	18·5	0·368
Multan . .	10·7	33·4	1·182	1·70	99·9	5·8	26·5	21·6	...
124 . . .	10·4	31·6	1·196	1·86	97·3	4·2	29·5	18·5	0·370
9-2028 . .	10·3	26·7	1·370	1·82	98·3	7·3	29·5	25·4	..
8-0889 . .	10·7	27·1	1·659	1·84	91·1	11·5	43·5	29·7	0·667
10-3651 . .	10·8	26·7	1·405	1·64	96·4	5·5	39·5	23·8	..
S 18 . .	10·7	32·9	1·266	2·02	99·2	7·0	25·5	22·6	0·430
Special No. 8 .	10·4	26·6	1·305	1·92	96·8	4·2	26·5	18·4	..
			2-rowed Barleys.						
2-0297 . .	10·2	35·6	1·551	1·86	99·2	4·0	31·5	17·4	0·488
3-0121 . .	10·4	35·9	1·576	1·48	100·8	4·0	33·5	18·2	0·495
1-0624 . .	10·1	53·2	1·910	1·70	97·3	8·0	54·5	20·2	0·569
Special 2-rowed .	10·4	44·5	1·578	2·16	99·3	5·3	31·5	20·7	0·455
Goldthorpe .	10·3	40·2	1·811	2·14	98·3	6·3	36·0	20·6	0·532
Carter's Prolific .	10·8	42·2	1·869	2·04	96·5	6·5	36·5	19·4	..

REPORT ON BARLEYS RECEIVED FROM CEREALIST TO GOVERNMENT, PUNJAB, FEBRUARY, 1930, AND ON THE MALTS EXPERIMENTALLY MADE FROM THEM.

Valuer's Reports.

Barley	Barleys	Malts	Malting Loss, per cent. on dry Barley	Extract lb. per 448 lb. Barley	Extract lb. per quarter on dry Malt
Cross N/R	Similar to commercial types of Karachi barley shipped in the past. Value according to market. Small demand for brewing, considerable demand for distilling.	Justify barley report. Note should be taken of low D. P. of S. 185 as grain distillers usually require more.	7·8	109·0	98·7
S 185			8·7	106·5	97·7
Rewari	Would always find a market at price above the average well worth growing on commercial basis.	Justify barley report. As big as Californian.	7·4	111·3	100·9
155-B	Another unusual barley of larger size than commonly received. It is not so well filled as Rewari but it might, by some customers, be preferred for its brighter colour. The valuers preferred 155-B and consider that there would be no point in growing Rewari if it could be grown up to sample.	Despite high nitrogen has remarkably good appearance. Bright malt.	9·3	99·4	91·5
152	Not unlike barleys shipped in the past from certain Indian districts. Quite useful for commercial purposes, especially distilleries.	Justify barley report.	9·9	107·0	99·2
152 1926-7			8·8	107·4	98·7
7-0315	Colour would always make it attractive. It might fetch a higher price than that ruling for Indian barley on account of its attractive appearance. Committee consider this barley well worthy of cultivation.	Justify barley report. Bright appearance. Note abnormally low diastatic power of 7-0315.	7·0	105·1	95·0
122	Of similar type but not so attractive.		10·0	102·5	95·4

Valuer's Reports—contd.

Barley	Barleys	Malts	Malting Loss, per cent. on dry Barley	Extract lb. per 448 lb. Barley	Extract lb. per quarter on dry Malt
Lyallpur	Similar to qualities of good record known in the past, but these are probably superior.	Justify barley report.	9·0	108·7	100·0
S 83			9·8	107·8	100·1
S 60	Similar, but smaller.		8·8	105·6	97·3
155a	Another type, known in the past which made very useful malt, and was always in demand by distillers and, to a certain extent, by some brewers.	Justify barley report except 155a which has very high N., low extract, and high D. P., and is a distillers' malt.	10·0	100·3	93·2
143			8·9	108·3	99·3
S-7/1			8·4	108·9	99·2
Mianwali .			7·8	109·3	99·5
Simla .	A group of very small barleys, but considerably brighter than that usually imported.	Justify barley report. 4-0303 not up to quality of others. Contains some immature unfilled corns.	9·5	105·9	98·0
Gujrat .			8·9	104·3	95·6
4-0303			10·3	100·9	93·8
Naushera .	Probably attractive to distillers. If malting test prove satisfactory, handicap of size might not prove serious.		9·0	105·0	96·6
Hoshiarpur			9·5	106·6	98·2
Gujarkhan			9·3	104·5	96·4
Lyallpur B			8·0	106·5	97·2
			9·4	105·4	97·2
Naushera Common	Rather larger than the last group, but the same remarks apply.	Justify barley report.	8·8	104·0	95·4
6-099			9·4	104·8	96·6
Cape B. S.			9·2	104·1	96·0
S 35/1			8·6	106·8	97·6

Valuer's Reports— contd.

Barley	Barleys	Malts	Malting Loss, per cent. on dry Barley	Extract lb. per 448 lb. Barley	Extract lb. per quarter on dry Malt
S/65	Thin barleys probably deficient in extract, but this must be tested by malting. Might not compete with other Eastern barleys.	Justify barley report. So small that they would not find a market. White appearance is attractive, but extract is very low, and nitrogen very high; would be practically unsaleable.	12·5	96·9	92·8
S/66			11·4	98·4	92·9
5-0577			9·5	94·4	87·4
Baluchistan	Quite useful barleys. Colour unusually bright.	Justify barley report. Multan low N., good extract, moderate D. P. Is the best in appearance.	9·0	104·8	96·1
Multan .			8·6	108·7	99·9
124			9·2	105·6	97·3
9-2028			9·9	106·1	98·3
8-0889	Too thin to be of any value.	Malt agrees, no value .	15·0	99·2	91·1
10-3651	Ditto . .	Ditto . .	10·0	103·1	96·4
S/18	Not so nice looking as Special No. 8 though similar.	Despite small size gave good extract.	8·9	107·7	99·2
Special No. 8.	Very nice ripe barley, but too thin and small for commercial use.	Ditto . .	10·3	103·8	96·8
2-0297	Most conformable to 2-rowed barley of home and foreign countries of which Committee have experience.	Compare very closely with best English malt. Extract of malt 2 lb. lower than English, but 5 or 6 lb. higher when referred back to barley on account of the lower moisture in the latter.	9·7	107·3	99·2
3-0121			9·5	108·9	100·8
1-0624	Quite an unusual sample, very large, but not filled out.	Unusual sample, high N. and high D. P. Low extract. Not a malting barley, and not suitable for import in competition with English.	9·0	106·1	97·3

Valuer's Reports—concld.

Barley	Barleys	Malts	Malting Loss, per cent. on dry Barley	Extract lb. per 448 lb. Barley	Extract lb. per quarter ondry Malt
Special 2-rowed.	Resemble large unripe Hanna. Appearance below 2-0297 and 3-0121.	Value as malt equal to Mid-European, but not so well filled. Would probably be an opening for this, and might replace imported European.	10·2	106·6	99·3
Goldthorpe			9·3	106·8	98·3
Carter's Prolific.	Poorest of all 2-rowed, and not promising from appearance.	Justifies barley report. Low extract, high N., poor appearance.	8·4	105·0	96·5

H. Lloyd Hind.

Enzymic activity of the malts.

The following determinations of liquefying and amylolytic power were made by L. Fletcher and J. B. Westwood to compare the enzymic activity of the experimentally-made Indian malts with that of commercial malts. The starch liquefying power was determined by the method of S. Jozsa and H. C. Gore, as modified by Fletcher and Westwood (*The Journal of the Institute of Brewing*, 1930, 550), the amylolytic and dextrinolytic powers according to the method described elsewhere in this issue (see p. 470). A standard starchpaste was used in the substrate in the determination of liquefying power, which is expressed as the weight of starch liquefied by the weight of malt taken.

The amylolytic and dextrinolytic powers are expressed by the weight of maltose produced by 1 grm. malt in one hour at 40°C. from soluble starch and J. L. Baker's α-amylodextrin respectively. The method of expression for amylolytic activity cannot be exactly compared with that conventionally employed in the standard method for determination of diastatic power on account of the different experimental conditions. Comparison of the figures in the following table with those given for diastatic power on page 464 indicates the approximates relationship :—

Amylolytic power×5·3=Diastatic power, Lintner.

The weight of maltose produced from α-amylodextrin, under the conditions prescribed, by 1 grm. malt in 1 hour at 40°C. is considered to represent the relative dextrinolytic activity of the malts.

The results indicate that the Indian malts examined have enzymic activities comparable with those of normal commercial malts.

Malts from Indian Barleys.

Samples from Rothamsted.

Variety	Liquefying power: Grms. starch liquefied by 1 grm. malt in 1 hour at 21°C.	Amylolytic power: Maltose produced by 1 grm. malt in 1 hour at 40°C. from	
		Starch	Dextrin
Baluchistan	110	4·9	2·2
Gujrat	102	5·1	3·0
5-0577	96	6·9	2·3
122	108	4·9	3·0
Cross N/R	176	6·4	2·7
S.60	110	5·9	3·2
6-099	148	6·1	3·7
155A	202	12·1	2·5
1-0624	180	10·3	2·0
2-0297	132	8·4	2·8
Goldthorpe	202	6·9	2·1
Carter's Prolific	176	5·3	2·2

Malts from Commercial Barleys.

Scotch	204	5·6	3·6
Ditto	182	7·9	4·8
Ditto	144	5·4	4·1
California	112	5·7	3·3
Ditto	160	5·7	—
Syrian	142	8·2	—
Ouchak	130	8·6	—
Australian Cape	130	5·2	—
Indian	94	9·3	—
Karachi	136	8·2	—

ABSTRACTS.

The expressed sap of corn plants as an indicator of nutrient needs. A. N. PETTINGER. [Reprinted from the *Journal of Agricultural Research*, Vol. 43, No. 2, July 15, 1931.]

The investigations reported here were undertaken to determine (1) whether certain characteristics of the expressed sap of corn plants are related to fertilizer practices, and if so, whether the relationships are sufficiently strong to be used as a basis for recommending the application of various fertilizers, and (2) to determine the extent of agreement between the results obtained and those obtained by other methods.

The saps were extracted with a small laboratory hydraulic press at a pressure of 6,500 pounds per square inch from 15-inch sections of stalks immediately above the ground.

The quantity of sap that can be extracted from the stalk issues of corn plants is closely related to diameter of stalk and to soil productivity. Large stalks produced on fertile land yielded from 70 to 90 c.c. of sap per stalk section, while small stalks, from unproductive soil, yielded only 20 to 30 c.c. per section. Large stalks contain more sap per unit of stalk volume than do small stalks.

There is a general relationship between the color of the expressed saps and the productiveness of the soil on which the plants are grown. Saps that are colorless after clarification, and those that are light brown in color indicate a fertile soil, while a dark-brown color in the sap is associated with unproductiveness.

The color of the expressed saps is closely correlated with potassium fertilization. Dark-brown saps were obtained from plants grown on soils that are deficient in available potassium, while colorless saps and those of the light shades of brown were obtained from plants grown on soils containing an abundance of available potassium.

The concentration of nitrate nitrogen showed only a fair degree of correlation with nitrogen fertilization. It was well correlated, however, with the soil supply of nitrate nitrogen.

The total phosphorus content of the sap is closely related to phosphatic fertilization. Applications of either superphosphate or farm manure increased the total phosphorus content from two to five times that contained in saps coming from plants grown on plots that received no phosphorus. Rock phosphate was less effective in increasing the phosphorus content than either superphosphate or manure.

Phosphorus concentrations were high when grain production was subnormal, and lower when grain production was normal to supernormal.

Because of the lessened demand for phosphorus after the ear has developed, phosphorus accumulates in the sap during September. The increase is approximately 100 per cent. and occurs irrespective of phosphate fertilization.

The potassium content of the sap shows nearly perfect correlation with potassium fertilization. The saps coming from plants grown on plots which received either muriate of potash or manure contained from two to sixteen times more potassium than saps from plants grown on similar plots receiving no such treatment.

There is a slight tendency for potassium to accumulate in the sap during the month of September. The increases, however, are small compared to those of phosphorus for the same period.

The following approximate nutrient concentrations in the sap are suggested tentatively as standards in diagnosing soils: Very deficient, nitrate nitrogen less than 100 p. p.m., total phosphorus less than 0·10 mgm. P_2O_5 per cubic centimeter of sap, potassium less than 1 mgm. K_2O per cubic centimeter; moderately deficient, nitrate nitrogen 200 p. p.m., total phosphorus 0·10 to 0·20 mgm. P_2O_5 per cubic centimeter, potassium 1·5 to 2·0 mgm. K_2O per cubic centimeter; ample, nitrate nitrogen above 300 p. p.m., total phosphorus above 0·20 mgm. P_2O_5 per cubic centimeter, and potassium more than 2 mgm. K_2O per cubic centimeter.

Although the concentration of nutrients in the sap is a good indicator of the relative quantities removed from the soil, there is a tendency to overestimate the removal on unproductive soils and to underestimate the removal on fertile soils.

The hydrogen-ion concentration of the expressed sap shows a fair correlation with potassium fertilization. In general, saps from plants grown on plots receiving potassium are below pH 5·45, while those from plots receiving no potassium are higher.

The hydrogen-ion concentration is not appreciably correlated with soil productivity.

The depth of green color shown by corn plants is a good indicator of nitrogen needs. The color of the plant, the results obtained by the Hoffer stalk test for nitrates, and the nitrate concentration in the sap all agree in indicating nitrogen needs in the tests conducted.

Dead tissues around the margins and between the veins of the leaves is an accurate indicator of potassium deficiency. This condition was well correlated with the Hoffer test for iron accumulations in the nodal tissues of the plant and small amounts of potassium in the sap. The absence of dead tissues in the leaves was accompanied by nodal tissues free from discolorations and iron accumulations and by large quantities of potassium in the sap.

Except in seasons which promote shallow root development, the amount and severity of lodging may be used as a guide to potassium needs.

Applications of potassium were accompanied by increased root anchorage. This was probably due to the prevention of iron accumulations at the nodes which allowed normal translocation of foods to the roots and therefore more extensive root development. (Author's Summary.)

Further studies on tobacco ring-spot in Virginia. R. G. HENDERSON and S. A. WINGARD. [Reprinted from the *Journal of Agricultural Research*, Vol. 43, August 1, 1931.]

Ring-spot, a virus disease of tobacco, has been reported from a large number of important tobacco-growing districts in the United States, Australia, Sumatra, South Africa, and Nyasaland.

Natural infection has been reported of sweetclover, yellow iron-weed, petunia, and squash with viruses which produce ring-spotlike symptoms when transferred to tobacco. These viruses differ from the tobacco ring-spot virus principally in the difference in the severity of the symptoms which they produce on tobacco. It is suggested that these viruses may possibly be attenuated forms of the tobacco ring-spot virus.

Jimson weed and cantaloupe have been found to be natural hosts of the tobacco ring-spot virus.

The ring-spot virus did not survive the winter in the roots of poke-weed plants.

The thermal death point of the ring-spot virus lies between 60° and 70° C.

Expressed juice from ring-spot-infected tobacco plants stored at a subzero temperature remained infectious for more than 22 months.

The ring-spot virus is very readily inactivated by desiccation.

The ring-spot virus can be precipitated and separated from expressed juice with either alcohol or acetone and recovered in water without any appreciable injury to its infective properties. The virus is filterable through a Berkefeld filter of W grade if the infectious juice is first freed of its suspended solid matter.

The ring-spot virus is infectious in dilutions as great as 1 to 1,000, but only a trace of infection has been obtained with 1 to 10,000 dilutions.

It has been found that under greenhouse conditions the ring-spot virus will persist for more than a year in the juice of tobacco plants which have been propagated by cuttings. The ring-spot symptoms may remain masked during this entire period.

As a result of artificial inoculation, typical ring-spot symptoms developed on detached tobacco leaves and cuttings kept alive in moist chambers. Artificial inoculation also showed tobacco leaves of intermediate age to be more susceptible to ring-spot infection than either the very old or the very young leaves.

Tobacco ring-spot infection was produced on tomato plants by grafting.

Very little evidence has been found in these studies to indicate that the ring-spot virus is seed borne in tobacco, but it was found to be very readily transmitted through the seed of petunia.

Artificial inoculation of certain varieties of potato with the ring-spot virus resulted in local infection on the leaves. Nevertheless, the virus occurring naturally in apparently healthy potato plants is regarded as being entirely different from the tobacco ring-spot virus.

Effect of ginning methods on cotton fibre quality studied. [Extract from *The Official Record*, United States Department of Agriculture, Vol. 10, No. 32, dated Washington, the 8th August, 1931.]

Ginning may seriously affect the grade and staple of cotton that contains too much moisture, according to studies made at the cotton ginning laboratory at Stoneville, Miss., and in the cotton fibre research laboratories at Washington, D. C. Too rapid operation of the gin will also injure the fibre. In explaining these observations, F. L. Gerdes, fibre technologist of the Bureau of Agricultural Economics, says, "Studies of the effects of ginning methods were begun as a result of protests received by the department from the cotton industry in this country and abroad to the effect that the preparation of American cotton is not as good as it used to be; that is, the cotton is rougher and more neppy and nappy than formerly."

Preliminary studies, Mr. Gerdes states, indicate that successful cleaning and extracting depend on the moisture content of seed cotton. Excessive moisture causes, among other things, a loss or shortening of fibre lengths and a lowering of grade. It also affects the preparation.

Inferior preparation of cotton in the Delta of the Mississippi, Mr. Gerdes explains, is usually the result of ginning early, green, sappy cotton, and late rainsoaked cotton without proper conditioning, and of operating the gins at full capacity on long-staple cotton. Even if a long-staple cotton has a so-called normal moisture content, preparation may be inferior if it is ginned too fast.

By improved machines and methods, the fibres of samples are sorted according to length. A comparison of the weight percentages of the different fibre lengths of samples ginned in various ways, Mr. Gerdes states, will show the best ginning conditions for any given cotton. Results obtained by measuring the color of cotton, he says, can be converted into terms of grade.

ORIGINAL ARTICLES.

THE OSMOTIC AND SUCTION PRESSURES OF THE RICE PLANT, *ORYZA SATIVA*, L., WHEN TREATED WITH DIFFERENT SALTS: A METHOD OF DETERMINING THE SALT REQUIREMENTS OF PLANTS.

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INTRODUCTION.

As rice forms one of the staple foodstuffs of a large part of the human population, serious attempts have been made to improve the quality of rice and to increase the yield per acre in a number of different ways. One of the main lines of investigations which is pursued by the agriculturists in all the rice-growing countries is the study of the effect of the various salts, supplied in the form of fertilizers, on the growth of the rice plant and on its yield. This line of investigation has proved profitable.

It is now known from the investigations carried out by Kellner [1884], Nagaoka [1904], Kelley [1911] and Kelley and Thompson [1910], that nitrogen is best absorbed by the plant in the form of ammonium sulphate in the early stages of growth of the rice plant and nitrates in the later stages of growth of the rice plant and nitrogen is an important element. The effect of iron in the form of ferric phosphate was studied by Giles and Carrero [1915], and it was found that the plant needs and absorbs very little iron. Similarly magnesium was tried and it was also not found of much use to the growth of the plant. Espino [1920] carried out a very carefully planned series of experiments to determine the salt requirements of the young rice plants, and he found that the best result was obtained with a four-

salt solution containing a trace of ferric phosphate, mono-potassium phosphate, calcium nitrate, magnesium sulphate and ammonium sulphate, having a total osmotic value of 0·08 atm. Similar experiments were tried by Trelease [1920] by making use of a three-salt solution and found that three fertilizers, potassium sulphate, calcium phosphate and ammonium sulphate in a certain concentration (his solution No. RIC 8) had the best effect on the growth of the rice plant.

From what is mentioned above, it could be seen that a large amount of useful information on the nature and quantities of salts necessary for the healthy growth of the rice plant is now available and the above workers have shown the importance of potassium, calcium, phosphorus and nitrogen to the rice plant.

Sahasrabuddhe [1928] in his experiments on the assimilation of nutrients by the rice plant has shown that the rice plant absorbs from the soil 28 lbs. of nitrogen, 20 lbs. of phosphoric acid, 60 lbs. of potash and 28 lbs. of lime when 2,000 lbs. of grain and 2,000 lbs. of straw are taken as the yields per acre. It clearly shows that these substances are absorbed from the constituents of the soil.

In all the investigations described above, no attention is paid to the osmotic relations of the plant with the culture solutions in which they are kept. It is necessary to know the changes produced in the osmotic and suction pressures of the rice plant when grown in culture solutions of different compositions and different concentrations. Ursprung and Blum [1921] from a series of experiments with *Vicia faba*, L. have shown that the suction pressure of roots becomes equal to the osmotic pressure of the surrounding medium. They transferred the roots from saw dust to cane sugar solutions of different concentrations and it was found that the suction pressure gradually changed and finally (if the solution was not strong) became equal to the osmotic pressure of the external solution. If the roots were transferred to a cane sugar solution of very high osmotic pressure, the absorbing zones of the roots died and new roots developed having the same suction pressure as that of the external solution. The above results obtained by Ursprung and Blum [1921] could be explained according to known facts. They used cane sugar which is not permeable to living cells, and it is expected that an interchange of water would take place till the pressures inside and outside are equalized. When they used strong solution, the living cells died on account of strong plasmolysis.

It was also shown by Demidenko [1926] that the osmotic pressure of the cell sap depended upon the osmotic pressure of the salt solution outside and an increase in the osmotic pressure of the solution in the soil increased the osmotic pressure of the cell sap. Similar observations were made by Litwinow [1926]. He also found the variations in the osmotic pressure of the culture solution of different concentrations and the suction pressures were proportional to the increasing concentration of the culture solution.

In the above observations of Demidenko [1926] and Litwinow [1926] on the relations that exist between the osmotic pressures of the plant cells and of the external solution, the changes produced in the osmotic pressure by the entry of ions of a salt are not taken into consideration. Some ions are absorbed more than the others and they may have some effect on the osmotic and suction pressures of plant cells, even when the osmotic values of different culture solutions used are kept the same as was done by Gericke [1930]. If the ions absorbed change the osmotic and suction pressures of the plant cells, the plants grown in culture solutions of different compositions but of the same osmotic value will have different osmotic and suction pressures, as some culture solutions contain ions which are absorbed by the plant, while other culture solutions have the ions which are not absorbed by it. Hence the osmotic relations of the plant with the surrounding medium, whether it be a culture solution or the soil, constantly vary and at no time is the osmotic pressure or suction pressure of the plant in equilibrium with the external medium. From what is said above, it would be of interest to study the osmotic and suction pressures of the rice plant when grown in different concentrations of salts and note the changes produced in them when the plant is grown in different concentrations of a salt which is known to be absorbed by the plant as compared to the changes produced when it is grown in similar concentrations of a salt which we know is not absorbed by it at a certain stage of growth.

Secondly in all the investigations summarised above, the influence of the omission of certain salts on growth and the amounts of the salts absorbed are determined by finding out the yield of grain and straw (per acre) after a particular treatment is given or making the chemical analysis of the plants at different stages of growth. The process of determining the chemical composition of the plants at different stages is both lengthy and tedious. For determining the total yield of the seeds and straw it is necessary to wait till the harvest time. For these reasons, if a simpler method of determining whether a plant is able to absorb a particular salt or not is devised, it should prove of great value to agriculturists and render easy their task of finding out the nature of salts absorbed by the plant and consequently needed by it. Whether it is possible to devise such a method can be seen from the arguments advanced below.

The osmotic pressure of a cell depends upon the presence of certain osmotically active substances in the cell contents and the force with which the water from outside passes into it depends upon the difference between the osmotic pressure of the external medium and the osmotic pressure of the cell contents minus the inwardly-directed wall pressure. So the absorption of water depends upon the suction force of the cell. It is also known that the plasmatic membrane which is impermeable to all substances, is permeable to some and consequently the passage

of the ions of the salts takes place from outside into the interior of the cell. The entry of a salt should disturb, even temporarily, the osmotic value of the cell and would in all probability, tend to increase its osmotic pressure. If on the contrary the salts or their ions are not absorbed or absorbed to a slight degree, no or little change in the osmotic value of the cell is expected. So the rise in the osmotic pressure of a cell when treated with a salt would indicate its absorption and *vice versâ*. It is also possible that the change in the osmotic value of the cell would also depend upon the concentration of the absorbed salt outside, and differences in the concentrations of the salt would lead to differences in the osmotic values. Thus the relationship between the concentrations of salts in the external solutions and the osmotic pressure of a plant cell could be established and the determination of the molecular concentration of the salt that would raise the osmotic value to its highest value would be possible. If these relations are known in the case of an economic plant like the rice, the amount of a fertilizer to be added to the soil to ensure its maximum absorption at any stage could be known without undergoing the lengthy process of making the chemical analysis of the plant.

It is not possible, except in a few cases, that the entry of an ion in a cell can be detected by microchemical tests and therefore, it is not possible to demonstrate the entry of an ion in a cell whose osmotic value has increased. But the absorption of an ion from the external solution can be shown by analysing chemically the external solution before and after the plant is kept in it. If chemical analysis shows that the salt is absorbed and if measurement of the osmotic pressure of the plant after immersion indicates a rise, the latter effect may safely be attributed to the former cause. If no rise in the osmotic pressure is detected when the salt is not absorbed, it would also support the same conclusion. It is also possible that the rise in the osmotic value of a cell may be temporary and may be followed by a fall. This is to be expected as, after the absorption of a salt, it may combine with other substances and thus lose its osmotic properties.

Lastly if the different concentrations of a salt that is absorbed by a cell raise its osmotic pressure to different degrees it can be concluded that the differences in the osmotic pressures of the cell contents are caused by the differences in the amounts of the salt absorbed. Dastur and Baptista [1931] have recently measured the osmotic and suction pressures of the roots and leaves of the rice plant during the whole season and they also noted that the manuring of the rice plants in the month of September by a dose of ammonium sulphate raised the osmotic and suction pressures of the cells of the roots and leaves in comparison with unmanured plants, though after a few days the osmotic and suction pressures of the manured plants dropped down and became normal again.

It seemed very likely from the reasoning advanced above and from the experiments of the abovementioned workers especially from the observations made by Dastur and Baptista [1931], that the investigation would yield interesting and in all probability profitable results.

The investigation would also be interesting from the point of view of the entry of the solutes into the cells. Various methods have been described by different workers to determine the permeability of the vegetable cells to the salts. Stiles [1924] has described fourteen different methods for the absorption of different substances in solution. The permeability of vegetable cells to the solutes could also be determined by measuring the osmotic and suction pressures before and after the immersion of the cells in known molecular solutions of different substances which are osmotically active.

It is proposed to carry out this investigation with the rice plant as the working material and if it proves profitable, to extend it to other plants also. The experiments described in this paper were first restricted to the rice plant as it was considered more important to perform an exhaustive series of experiments with one plant than to obtain incomplete data with a number of plants. The observations were then extended to other plants in order to support the conclusions arrived at by the studies with the rice plant.

Investigation.

As mentioned in the introduction above, the rice plant absorbs nitrogen in the form of potassium nitrate in the later stages and as nitrogen is such an important element in the foodstuffs of plants, it is undertaken first to measure the osmotic and suction pressures of the rice plant at different stages of growth when treated with different strengths of these substances either in pure form or in combination with others in culture solutions. It would also be of interest to study the changes in the osmotic and suction pressures of plants when treated with different salts such as monopotassium phosphate, calcium sulphate and potassium sulphate as K, P, and Ca are now known to be absorbed and assimilated by the plant.

Method.

Rice seeds (Kolamba variety No. 42) were sown in pots and eight days after sowing, they were transferred to the solution of ammonium sulphate of nine different strengths, from N/100 to N/900 molecular solutions and kept in the solutions for eight days. At the end of the eighth day, *i.e.*, 16th day after sowing, the osmotic and suction pressures of the seedlings were measured. The solutions were kept in

museum jars of one litre capacity for which wooden lids fitting to them with twelve holes to hold the seedlings were specially prepared. The seedlings from saw-dust were transferred on the eighth day to a solution of ammonium sulphate, one seedling was fixed in each hole on the lid. The lid was completely sealed off by paraffin wax. Necessary precaution was taken to avoid the growth of moulds in the culture solution on the inside of the lid and the collar of the plants.

The osmotic pressure of the plant was determined by the ordinary method of plasmolysis of De Vries [1884] and by the plasmometric method of Hofler [1918] for the same reason as stated by Dastur and Baptista [1931].

The osmotic pressure of the roots and leaves were determined in order to have an idea of the osmotic pressure existing in the plant body. In the case of roots osmotic pressure was determined near the apex and the base and in the case of leaf in the leaf sheath and the leaf lamina below the apex. The same procedure as was adopted by Dastur and Baptista [1931] in determining the osmotic pressure was followed here.

The suction pressure of the rice plants after similar treatment with salts was measured, as this would give indirect evidence of the absorption or non-absorption of salts. The absorption of a salt changes the osmotic value of the cell contents and consequently the suction pressure of the cell also alters.

The method of measuring the suction pressure of cells devised by Ursprung and Blum [1916,3] and modified several times by the same authors is used in this investigation.

According to the method devised by Ursprung and Blum [1923] and modified by Molz [1926] a properly trimmed strip of a tissue is placed on a glass slide marked with a scale of five centimeters and subdivided into millimeters. The length of the tissue in paraffin oil was measured. The oil was removed from the strip of tissue which was kept in a glass stoppered bottle containing the sugar solution. The solution in which the strip of tissue suffered no change in length was found out. The osmotic pressure of the solution was equivalent to the average suction pressure of the strip of the tissue.

The following table gives the value of osmotic pressure of the roots of seedlings fifteen days old after sowing of the seeds and kept on the eighth day in solutions of different strengths of ammonium sulphate for eight days. The period of eight days for keeping the seedlings in the solutions was specially selected as by chemical analysis of the external solution it was found that no salt is absorbed after eight days owing to the equilibrium between the external and internal concentrations of the salt having been reached. The osmotic pressure measured on the eighth day at the root apex was 3·6 atms. and at the root base 4·4 atms. In all the tables the osmotic values of the external solutions were obtained by calculations from the

tables given by Adie [1891] who determined the osmotic pressures of various solutions of known strengths by means of Pfeffer's method.

TABLE I.

The osmotic pressure of the roots of seedlings 7 days old kept in $(NH_4)_2SO_4$ solution for eight days.

Date	Strength of the $(NH_4)_2SO_4$ solution	O. V. of the external solution in atms.	O. P. at root apex in atms.	O. P. at root base in atms.
25th October 1929 . . .	N/100	0·528	3·60	4·95
18th December 1929 . . .	N/200	0·264	5·70	7·50
10th November 1929 . . .	N/300	0·176	6 20	8·80
23rd December 1929 . . .	N/400	0·132	4·95	7·00
,, ,, 1929 . . .	N/500	0·106	2·50	3·60
4th January 1930 . . .	N/600	0·088	3·60	4·95
6th ,, 1930 . . .	N/700	0·075	3·20	4·95
26th ,, 1930 . . .	N/800	0·066	2·50	2·50
28th ,, 1930 . . .	N/900	0·059	2·50	2·50

Similarly the osmotic pressure in leaves of seedlings kept in $(NH_4)_2SO_4$ solution on the eighth day after sowing was measured after eight days.

The osmotic pressure on the eighth day (before the seedlings were transferred to solutions) was 6·2 atms. at the leaf sheath and 8·8 atms. at the leaf apex.

TABLE II.

The osmotic pressure of the leaves of seedlings 15 days old.

Date	Strength of the $(NH_4)_2SO_4$ solution	O. V. of the external solution in atms.	O. P. at leaf sheath in atms.	O. P. at leaf apex in atms.
25th October 1929 . . .	N/100	0·528	6·20	11·50
18th December 1929 . . .	N/200	0·264	8·80	16·10
10th November 1929 . . .	N/300	0·176	11·50	15·90
23rd December 1929 . . .	N/400	0·132	8·80	11·50
,, ,, 1929 . . .	N/500	0·106	5·65	7·00
4th January 1930 . . .	N/600	0·088	6·20	7·50
6th ,, 1930 . . .	N/700	0·075	4·95	7·50
26th ,, 1930 . . .	N/800	0·066	3·66	4·95
28th ,, 1930 . . .	N/900	0·059	2·50	3·60

The Tables I and II clearly show that the osmotic pressure in the roots and leaves rises as the concentrations of the ammonium sulphate solution decrease from N/100 to N/300. The highest values of the osmotic pressure in the roots and leaves are obtained when the seedlings are kept in the N/300 $(NH_4)_2SO_4$. They are 8·8 atms. in the root base and 15·9 atms. at the leaf apex. The osmotic pressures of the seedlings kept in solutions of lower strengths than N/300 begin decreasing as the dilution of the solution increases. (After N/800 $(NH_4)_2SO_4$ the osmotic pressure remains more or less uniform.) N/300 $(NH_4)_2SO_4$ produces maximum osmotic pressure and the lowest values of osmotic pressure are produced when N/800 $(NH_4)_2SO_4$ is used.

On comparing the values of osmotic pressures of roots and leaves of the seedlings determined before they were transferred to the solutions of ammonium sulphate, with the values of osmotic pressures of roots and leaves of the seedlings determined after they had remained in these solutions for eight days, it is seen that the osmotic pressures of the roots and leaves in solution of ammonium sulphate of strengths N/100 to N/400 have risen considerably, while in lower strengths of ammonium sulphate solutions the osmotic pressures have fallen.

Similar experiments were tried with seedlings fifteen days old after sowing and transferred to $(NH_4)_2SO_4$ solutions of different strengths. The osmotic pressure of the root was measured after the seedlings had remained for eight days in each experiment.

The osmotic pressure measured on the 15th day before the seedlings were transferred to the following solutions was 2·0 atms. at the root apex and 3·0 atms. at the root base.

TABLE III.

The osmotic pressures in the roots of seedlings 23 days old.

Date	Strength of the $(NH_4)_2SO_4$ solution	O. V. of the external solution in atms.	O. P. at root apex in atms.	O. P. at root base in atms.
2nd November 1929 . . .	N/100	0·528	3·60	6·20
26th December 1929 . . .	N/200	0·264	5·70	7·50
18th November 1929 . . .	N/300	0·176	7·50	11·50
31st December 1929 . . .	N/400	0·132	3·20	4·95

TABLE III—*contd.*

The osmotic pressures in the roots of seedlings 23 days old—contd.

Date.	Strength of the $(NH_4)_2SO_4$ solution	O. V. of the external solution in atms.	O. P. at root apex in atms.	O. P. at root base in atms.
31st December 1929 . . .	N/500	0·106	3·20	4·40
12th January 1930 . . .	N/600	0·088	3·60	3·60
14th ,, 1930 . . .	N/700	0·075	2·50	2·50
3rd February 1930 . . .	N/800	0·066	2·50	3·60
5th ,, 1930 . . .	N/900	0·059	2·50	3·60

Similarly the osmotic pressures of the leaves of the above seedlings were measured. Osmotic pressure at the leaf sheath was 5·0 atms. and at the leaf apex 6·2 atms. on the fifteenth day.

TABLE IV.

The osmotic pressure of the leaves of seedlings 23 days old.

Date	Strength of the $(NH_4)_2SO_4$ solution	O. V. of the external solution in atms.	O. P. at leaf sheath in atms.	O. P. at leaf apex in atms.
2nd November 1929 . . .	N/100	0·528	8·80	11·50
26th December 1929 . . .	N/200	0·264	9·50	11·50
18th November 1929 . . .	N/300	0·176	14·50	18·60
31st December 1929 . . .	N/400	0·132	6·20	8·80
,, ,, 1929 . . .	N/500	0·106	5·65	8·1
12th January 1930 . .	N/600	0·088	4·40	7·50
14th ,, 1930 . . .	N/700	0·075	5·00	6·20
3rd February 1930 . . .	N/800	0·066	4·40	4·95
5th ,, 1930 . . .	N/900	0·059	4·40	4·95

In the seedlings 23 days old the N/300 $(NH_4)_2 SO_4$ solution produces maximum osmotic pressure in the roots and leaves and the values of the osmotic pressure are higher than the corresponding values of osmotic pressure in the seedlings of fifteen days as can be seen from Tables I and II.

The suction pressures of the seedlings 15 and 23 days old after sowing the seeds, were measured after keeping them in the solution of ammonium sulphate of different concentrations for eight days before measurements were made. The suction pressure values should always be lower than the corresponding values of osmotic pressures on account of opposite wall pressure existing in the cells. This was found to be the case as the comparisons of the results above and below would show.

The following table gives the suction pressures of the roots of seedlings fifteen days old. The suction pressure on the eighth day was 1·3 atms. at the root apex and 2·5 atms. at the root base.

TABLE V.

The suction pressure of the roots of seedlings on the 15th day.

Date	Strength of the $(NH_4)_2SO_4$ solution	O. V. of the external solution in atms.	S. P. at root apex in atms.	S. P. at root base in atms.
25th October 1929 . . .	N/100	0·528	1·3	2·50
18th December 1929 . . .	N/200	0·264	3·6	4·95
10th November 1929 . . .	N/300	0·176	3·6	4·95
23rd December 1929 . . .	N/400	0·132	3·6	4·95
,, ,, 1929 . . .	N/500	0·106	1·3	2·50
4th January 1930 . . .	N/600	0·088	3·2	3·60
6th ,, 1930 . . .	N/700	0·075	2·5	3·60
26th ,, 1930 . . .	N/800	0·066	1·3	1·30
28th ,, 1930 . . .	N/900	0·059	1·3	1·30

The values of the suction pressures in the roots remain the same in N/200, N/300 and N/400 ammonium sulphate solutions, while in the solutions of other strengths they are lower. The constant suction pressure values in N/200, N/300 and N/400 ammonium sulphate solutions are probably due to increase of negative wall

pressure of the cells in greater degrees when immersed in N/200, N/300 and N/400 $(NH_4)_2SO_4$ solutions and that is responsible for the constant values of suction pressure in these solutions. Otherwise the results agree with the corresponding results of osmotic pressures.

The following Table VI gives the suction pressure of the leaves of seedlings after fifteen days. The suction pressure on the eighth day was 3·6 atms. at the leaf sheath and 4·95 atms. at the leaf apex.

TABLE VI.

The suction pressure of the leaves of seedlings on the 15th day.

Date	Strength of the $(NH_4)_2SO_4$ solution	O. V. of the external solution in atms.	S. P. at leaf base in atms.	S. P. at leaf apex in atms.
25th October 1929 . . .	N/100	0·528	3·60	7·50
18th December 1929 . . .	N/200	0·264	7·50	8·80
10th November 1929 . . .	N/300	0·176	8·80	13·00
23rd December 1929 . . .	N/400	0·132	7·00	8·80
,, ,, 1929 . . .	N/500	0·106	3·60	6·20
4th January 1930 . . .	N/600	0·088	4·95	6·20
6th ,, 1930 . . .	N/700	0·075	3·60	5·65
26th ,, 1930 . . .	N/800	0·066	2·50	3·60
28th ,, 1930 . . .	N/900	0·059	2·00	3·20

In N/300 ammonium sulphate solution the suction pressure is the highest in the leaves. The results agree with those of the osmotic pressure in the leaves.

The suction pressures of the seedlings 23 days old after sowing and kept in the ammonium sulphate solutions of different concentrations for eight days, on the 15th day after sowing were determined. The following Table VII gives the suction pressures of the roots. The suction pressure on the 15th day before the seedlings were

transferred to the following solutions was 2·5 atms. at the root apex and 3·6 atms. at the root base.

TABLE VII.

The suction pressure of the roots of seedlings 23 days old.

Date	Strength of the $(NH_4)_2SO_4$ solution	O. V. of the external solution in atms.	S. P. at root apex in atms.	S. P. at root base in atms.
2nd November 1929	N/100	0·528	2·50	3·60
26th December 1929	N/200	0·264	3·60	4·95
18th November 1929	N/300	0·176	3·60	6·20
31st December 1929	N/400	0·132	1·30	4·40
,, ,,	N/500	0·106	2·00	2·50
12th January 1930	N/600	0·088	2·00	2·50
14th ,, 1930	N/700	0·075	1·30	2·50
3rd February 1930	N/800	0·066	0·75	1·30
5th ,, 1930	N/900	0·059	2·00	2·50

The results of the suction pressures for 23 days old seedlings do not differ from those obtained for the 15 days seedlings.

The following table gives the suction pressures in the leaves of 23 days old seedlings. The suction pressure on the 15th day was 5·0 atms. at the leaf sheath and 6·2 atms. at the leaf apex.

TABLE VIII.

The suction pressure of the leaves of the seedlings 23 days old.

Date	Strength of the $(NH_4)_2SO_4$ solution	O. V. of the external solution in atms.	S. P. at leaf sheath in atms.	S. P. at leaf apex in atms.
2nd November 1929	N/100	0·528	4·95	8·80
26th December 1929	N/200	0·264	7·50	10·10
18th November 1929	N/300	0·176	8·80	15·90
31st December 1929	N/400	0·132	4·95	7·50
,, ,, 1929	N/500	0·106	3·60	7·00
12th January 1930	N/600	0·088	3·60	6·20
14th ,, 1930	N/700	0·075	3·60	5·00
3rd February 1930	N/800	0·066	2·00	2·50
5th ,, 1930	N/900	0·059	3·60	3·60

The results of the suction pressure for 23 days old seedlings agree very closely with those obtained for 15 days old seedlings and the value of the suction pressure is highest in the solution of N/300 ammonium sulphate as the value of the osmotic pressure in 23 days old seedlings is also the highest in the values obtained for the roots and leaves in all the experiments. The lower values of osmotic pressures and suction pressures in the roots in N/300 ammonium sulphate solution may be due to the absorption of ammonium and sulphate ions which are passed on to the cells in the leaf sheath and leaf lamina, while the very high values of the osmotic and suction pressures in the leaf sheath and leaf lamina may be due to the accumulation of the same ions in the cells. In the lower concentrations of the ammonium sulphate solutions the values of the osmotic and suction pressures in roots and leaves are not so widely divergent as the absorption of ammonium and sulphate ions is very little or *nil* and the differences between the values of both in the roots and leaves are small.

It is known that the rice plant absorbs very little of nitrogen in the form of nitrates and if the conception that the absorption of an ion or ions is accompanied with increase in the osmotic and suction pressures of cells be correct, the treatment of seedlings at the same stages of growth and kept under the same conditions with different concentrations of KNO_3 would not be accompanied by an appreciable rise in the osmotic and suction pressures of the roots and leaves. At the same time the values of the osmotic and suction pressures would also vary according to the concentration of the solution of KNO_3. Same types of experiments were done with the seedlings 15 and 23 days old with different concentrations of KNO_3, as were performed with ammonium sulphate solution. The plants after 8 and 15 days were kept in solution of KNO_3 for eight days and the osmotic pressures of the seedlings were measured. It should be pointed out that while the seedlings of rice of both ages remain quite healthy in all the concentrations of ammonium sulphate, the seedlings showed signs of death in higher concentrations of potassium nitrate. The measurement of the osmotic pressure of the seedlings in the higher concentrations of potassium nitrate was rendered difficult and in some cases no measurements could be made. For this reason it was necessary to use concentrations lower than N/900 of potassium nitrate. The concentrations of KNO_3 used were N/100 to N/1500 and in solution of each strength the seedlings of eight days old were kept for eight days. Similarly the seedlings of 15 days old were kept in each of the solutions of the above-mentioned strengths. So the rice seedlings had to be raised a number of times in order to perform a series of experiments described above and below and as they could not be done simultaneously, the slight variations in the results obtained were expected on account of the difference in weather and the dates of germination and these slight variations may lead to some discrepancies in the results. In spite of

these sources of errors the results so far obtained are highly concordant and conclusive.

The following table gives the osmotic pressure of the roots of the seedlings fifteen days old. The osmotic pressure measured on the eighth day was 3·6 atms. at the root apex and 4·4 atms. at the root base.

TABLE IX.

The osmotic pressure of the roots of seedlings on the 15th day.

Date	Strength of the KNO_3 solution	O. V. of the external solution in atms.	O. P. of the root apex in atms.	O. P. of the root base in atms.
3rd March 1930 . . .	N/100	0·376	2·00	2·50
,, ,, 1930 . . .	N/200	0·188	2·00	2·50
5th ,, 1930 . . .	N/300	0·125	2·50	2·50
9th ,, 1930 . . .	N/400	0·094	2·50	2·50
,, ,, 1930 . . .	N/500	0·075	2·50	2·50
28th ,, 1930 . . .	N/600	0·063	2·50	2·50
,, ,, 1930 . . .	N/700	0·054	2·50	2·50
23rd ,, 1930 . . .	N/800	0·047	2·50	2·50
,, ,, 1930 . . .	N/900	0·042	1·30	2·00
29th ,, 1930 . . .	N/1000	0·038	2·50	3·60
3rd May 1930	N/1100	0·034	2·50	3·60
27th April 1930	N/1200	0·031	2·50	3·20
27th ,, 1930	N/1300	0·029	2·50	3·20
27th ,, 1930	N/1400	0·027	2·50	3·20
3rd May 1930	N/1500	0·025	2·50	3·20

The results clearly indicate that the osmotic pressure remains the same in the roots in higher concentrations of potassium nitrate solutions, *i.e.*, from N/100 to N/900 and the osmotic pressure at the root base is slightly higher in the concentration of potassium nitrate solutions from N/1000 to N/1500.

The following table gives the osmotic pressure of the leaves of seedlings 15 days old. The osmotic pressure on the 8th day was 6·2 atms. at the leaf base and 8·8 atms. at the leaf apex.

TABLE X.

The osmotic pressure of the leaves of seedlings 15 days old.

Date	Strength of the KNO_3 solution	O. V. of the external solution in atms.	O. P. of the leaf sheath in atms.	O. P. of the leaf apex in atms.
3rd March 1930	N/100	0·376	3·60	4·95
,, ,, 1930	N/200	0·188	3·60	4·95
5th ,, 1930	N/300	0·125	3·60	4·95
9th ,, 1930	N/400	0·094	..	..
,, ,, 1930	N/500	0·075	..	..
28th ,, 1930	N/600	0·063	2·50	4·95
,, ,, 1930	N/700	0·054	4·95	6·20
23rd ,, 1930	N/800	0·047	2·50	3·60
,, ,, 1930	N/900	0·042	2·50	2·50
29th ,, 1930	N/1000	0·038	4·95	4·95
3rd May 1930	N/1100	0·034	4·40	4·95
27th April 1930	N/1200	0·031	3·60	4·40
,, ,, 1930	N/1300	0·029	3·60	4·95
,, ,, 1930	N/1400	0·027	3·60	4·95
3rd May 1930	N/1500	0·025	3·60	4·95

The results of the osmotic pressure in the leaves show some irregularities. They show maximum osmotic pressure in N/700 which is followed by a fall. The results in general terms show that the osmotic pressure at the leaf apex rises to 4·95 atms. while at the leaf base it reaches 3·6 atms. The results suggest very little absorption of $K^{\cdot}$ and NO_3' ions from the solutions. That this is the case can be seen from the

experiments performed with 23 days old seedlings. The osmotic pressure on the 15th day was 2·0 atms. at the root apex and 3·0 atms. at the root base.

TABLE XI.

The osmotic pressure of the roots of seedlings 23 days old.

Date	Strength of the KNO_3 solution	O. V. of the external solution in atms.	O. P. of the root apex in atms.	O. P. of the root base in atms.
11th March 1930 . . .	N/100	0·376	Plants died.	Plants died.
„ „ 1930 . . .	N/200	0·188	„ „	„ „
13th „ 1930 . . .	N/300	0·125	„ „	„ „
17th „ 1930 . . .	N/400	0·094	„ „	„ „
„ „ 1930 . . .	N/500	0·075	„ „	„ „
5th April 1930 . . .	N/600	0·063	2·00	3·60
„ „ 1930 . . .	N/700	0·054	2·50	2·50
31st March 1930 . . .	N/800	0·047	2·00	2·50
„ „ 1930 . . .	N/900	0·042	2·50	3·60
6th April 1930 . . .	N/1000	0·038	3·60	4·95
11th May 1930 . . .	N/1100	0·034	3·60	3·60
5th „ 1930 . . .	N/1200	0·031	3·60	4·95
„ „ 1930 . . .	N/1300	0·029	3·60	3·60
„ „ 1930 . . .	N/1400	0·027	3·60	4·95
11th „ 1930 . . .	N/1500	0·025	3·60	4·95

The results indicate that the plants absorb slight amounts of KNO_3 from N/1000 to N/1200 KNO_3 solutions and still lesser amounts from the lower strengths of KNO_3 than N/1200. From higher concentrations of KNO_3 the plants don't absorb KNO_3 at all and the values of osmotic pressure are the same as obtained for the seedlings 15 days old.

The following table gives the osmotic pressure of the leaves of seedlings 23 days old. The osmotic pressure on the 15th day was 5·0 atms. at the leaf base and 6·2 atms. at the leaf apex.

TABLE XIII.

The osmotic pressure of the leaves of seedlings 23 days old.

Date	Strength of the KNO_3 solution	O. V. of the external solution in atms.	O. P. at the leaf sheath in atms.	O. P. of the leaf apex in atms.
11th March 1930 . . .	N/100	0·376	Plants died.	Plants died
,, ,, 1930 . . .	N/200	0·188	,, ,,	,, ,,
13th ,, 1930 . . .	N/300	0·125	,, ,,	,, ,,
17th ,, 1930 . . .	N/400	0·094	,, ,,	,, ,,
,, ,, 1930 . . .	N/500	0·075	,, ,,	,, ,,
5th April 1930 . . .	N/600	0·063	3·60	6·20
,, ,, 1930 . . .	N/700	0·054	3·60	4·95
31st March 1930 . . .	N/800	0·047	2·50	3·60
,, ,, 1930 . . .	N/900	0·042	3·60	4·95
6th April 1930 . . .	N/1000	0·038	4·95	6·20
11th May 1930 . . .	N/1100	0·034	4·95	4·95
5th ,, 1930 . . .	N/1200	0·031	4·95	6·20
,, ,, 1930 . . .	N/1300	0·029	3·60	4·95
,, ,, 1930 . . .	N/1400	0·027	3·60	4·95
11th ,, 1930 . . .	N/1500	0·025	3·60	4·95

Again taking the results in a general way the highest values of osmotic pressures are obtained with solutions of KNO_3 of N/1000 to N/1200 strengths. Summarizing the results of the osmotic pressure of the roots and leaves of 23 days old seedlings in different concentrations of KNO_3 some facts of interest emerge from them. Firstly very little nitrates are absorbed from the potassium nitrate solutions and secondly, maximum absorption takes place from N/1000 to N/1500 KNO_3 solutions by the roots and leaves of 15 days old seedlings, and the same is the case with the seedlings 23 days old. The values of osmotic pressure in the leaves and roots of 23 days old seedlings are higher than the corresponding values of osmotic pressure of leaves and of 15 days old seedlings in N/1000 to N/1200 KNO_3 solutions. This indicates that

the amount of nitrates absorbed by the rice seedlings from the solutions of same concentrations increases as the age advances.

In all cases there is a fall in the osmotic pressure of the roots and leaves of the seedlings after they were transferred to KNO_3 solutions as compared with those existing in the seedlings before transfer. This was not the case in $(NH_4)_2SO_4$ solutions except in dilute solution.

The suction pressures of the roots and leaves of the rice seedlings of 15 days and 23 days old when grown in KNO_3 solutions of different concentrations for 8 days were also determined as was done when the plants were grown in ammonium sulphate solutions.

The following table gives the suction pressure in the roots of seedlings 15 days old and kept in different concentrations of KNO_3 for a week. The suction pressure on the 8th day was 1·3 atms. at the root apex and 2·5 atms. at the root base.

TABLE XIII.

The suction pressure of the roots of seedlings on the 15th day.

Date	Strength of the KNO_3 solution	O. V. of the external solution in atms.	S. P. at the root apex in atms.	S. P. at the root base in atms.
3rd March 1930 . . .	N/100	0·376	1·00	1·30
,, ,, 1930 . . .	N/200	0·188	1·00	1·00
4th ,, 1930 . . .	N/300	0·125	1·00	2·00
9th ,, 1930 . . .	N/400	0·094	..	..
,, ,, 1930 . . .	N/500	0·075	..	..
28th ,, 1930 . . .	N/600	0·063	1·30	2·50
,, ,, 1930 . . .	N/700	0·054	1·30	3·60
23rd ,, 1930 . . .	N/800	0·047	1·30	2·00
,, ,, 1930 . . .	N/900	0·042	1·30	2·00
29th ,, 1930 . . .	N/1000	0·038	1·30	2·00
3rd May 1930 . . .	N/1100	0·034	1·00	2·50
27th April 1930 . . .	N/1200	0·031	2·00	2·50
,, ,, 1930 . . .	N/1300	0·029	1·30	2·00
,, ,, 1930 . . .	N/1400	0·027	1·30	2·00
3rd May 1930 . . .	N/1500	0·025	1·30	2·50

The results show clearly that the values of suction pressure remains steady and no rise in the values is observed except with N/700 at the root base which may be due to reasons mentioned above. The following table gives the suction pressure of the leaves of 15 days old seedlings. The results are of the same type as those obtained for the roots. The suction pressure on the 8th day was 3·6 atms. at the leaf base and 4·95 atms. at the leaf apex.

TABLE XIV.

The suction pressure of the leaves of seedlings 15 days old.

Date	Strength of the KNO_3 solution	O. V. of the external solution in atms.	S. P. of the leaf sheath in atms.	S. P. of the leaf apex in atms.
3rd March 1930 . . .	N/100	0·376	2·00	3·20
,, ,, 1930 . . .	N/200	0·188	1·30	2·50
5th ,, 1930 . . .	N/300	0·125	2·50	4·40
9th ,, 1930 . . .	N/400	0·094	..	..
,, ,, 1930 . . .	N/500	0·075	..	..
28th ,, 1930 . . .	N/600	0·063	2·50	4·40
,, ,, 1930 . . .	N/700	0·054	3·60	5·65
22rd ,, 1930 . . .	N/800	0·047	2·00	2·50
,, ,, 1930 . . .	N/900	0·042	2·50	3·10
29th ,, 1930 . . .	N/1000	0·038	3·60	3·60
3rd May 1930 . . .	N/1100	0·034	2·50	4·40
27th April 1930 . . .	N/1200	0·031	2·50	3·60
,, ,, 1930 . . .	N/1300	0·029	2·00	3·60
,, ,, 1930 . . .	N/1400	0·027	2·50	3·60
3rd May 1930 . . .	N/1500	0·025	2·50	3·20

The measurements of the suction pressure of the seedlings twenty three days old and kept in different concentrations of potassium nitrate solutions for seven days. The following table gives the suction pressure of the roots of twenty three days old seedlings.

The results agree with those of the osmotic pressure of the roots of the seedlings of the same age. There is an indication of slight rise of suction pressure in

N/1000 to N/1500 potassium nitrate solutions. The suction pressure measured on the fifteenth day was 2·5 atms. at the root apex and 3·6 atms. at the root base.

TABLE XV.

The suction pressure of the roots of seedlings 23 days old.

Date	Strength of the KNO_3 solution	O. V. of the external solution in atms.	S. P. at the root apex in atms..	S. P. at the root base in atms.
11th March 1930	N/100	0·376	Plants died .	Plants died .
,, ,, 1930	N/200	0·188	,, ,, .	,, ,, .
13th ,, 1930	N/300	0·125	,, ,, .	,, ,, .
17th ,, 1930	N/400	0·094	,, ,, .	..
,, ,, 1930 . . .	N/500	0·075	..	..
5th April 1930	N/600	0·063	1·30	2·50
,, ,, 1930 . . .	N/700	0·054	1·30	2·00
31st March 1930	N/800	0·047	1·30	1·30
,, ,, 1930	N/900	0·042	1·30	2·00
6th April 1930	N/1000	0·038	2·50	3·60
11th May 1930	N/1100	0·034	2·50	2·50
5th ,, 1930	N/1200	0·031	2·50	3·20
,, ,, 1930	N/1300	0·029	2·50	3·60
,, ,, 1930	N/1400	0·027	2·50	2·50
11th ,, 1930	N/1500	0·025	2·50	2·50

The following table gives the values of suction pressure in the leaves of 23 days old seedlings. The suction pressure measured on the 15th day was 5·0 atms. at the leaf base and 6·2 atms. at the leaf apex.

TABLE XVI.

The suction pressure of the leaves of the seedlings 23 days old.

Date	Strength of the KNO_3 solution	O. V. of the external solution in atms.	S. P. at the leaf sheath in atms.	S. P. at the leaf apex in atms.
11th March 1930	N/100	0·376	Plants died .	Plants died .
„ „ 1930	N/200	0·188	„ „ .	„ „ .
13th „ 1930	N/300	0·125	„ „ .	„ „ .
17th „ 1930	N/400	0·094	..	..
„ „ 1930	N/500	0·075	..	..
5th April 1930	N/600	0·063	2·50	4·95
„ „ 1930	N/700	0·054	2·50	3·60
31st March 1930	N/800	0·047	1·30	2·50
„ „ 1930	N/900	0·042	2·50	4·95
6th April 1930	N/1000	0·038	3·60	4·95
11th May 1930	N/1100	0·034	3·60	4·95
5th „ 1930	N/1200	0·031	3·60	4·95
„ „ 1930	N/1300	0·029	3·60	3·60
„ „ 1930	N/1400	0·027	2·50	3·60
11th „ 1930	N/1500	0·025	2·50	3·60

The suction pressure shows a rise in N/1000 to N/1200 KNO_3 solutions as was the case in the seedlings 15 days old. The value of suction pressure rises in the leaf sheath from 2·5 atms. to 3·6 atms. and in the leaf lamina from 3·6 atms. to 4·95 atms.

The difference in the values of the osmotic pressure and the suction pressure when treated with the ammonium sulphate and potassium nitrate are very striking. The differences in the values could be seen at a glance from the following tables which give the values of osmotic pressures when treated with ammonium sulphate

side by side the corresponding values of the osmotic pressure when treated with potassium nitrate.

TABLE XVII.

The comparison of the osmotic pressures of the roots of seedlings grown in $(NH_4)_2SO_4$ *and* KNO_3 *solutions.*

Strength of the solution	Root apex		Root base	
	O. P. with $(NH_4)_2SO_4$ in atms.	O. P. with KNO_3 in atms.	O. P. with $(NH_4)_2SO_4$ in atms.	O. P. with KNO_3 in atms.
N/100	3·60	2·00	4·95	2·50
N/200	5·70	2·00	7·50	2·50
N/300	6·20	2·50	8·80	3·60
N/400	4·95	2·50	7·00	2·50
N/500	2·50	2·50	3·60	2·50
N/600	3·60	2·50	4·95	2 50
N/700	3·20	2·50	4·95	2·50
N/800	2·50	2·50	2·50	2·50
N/900	2·50	2·50	2·50	2·00

TABLE XVIII.

The comparison of the osmotic pressures of the leaves of seedlings grown in $(NH_4)_2SO_4$ *and* KNO_3 *solutions.*

Strength of the solution	Leaf sheath		Leaf apex	
	O. P. with $(NH_4)_2SO_4$ in atms.	O. P. with KNO_3 in atms.	O. P. with $(NH_4)_2SO_4$ in atms.	O. P. with KNO_3 in atms.
N/100	6·20	3·60	11·10	4·95
N/200	8·80	3·60	10·10	4·95
N/300	11·50	3·60	15·90	4·95
N/400	8·80	3·60	11·90	4·95
N/500	5·65	3·60	7·00	4·95
N/600	6·20	2·50	7·50	4·95
N/700	4·95	4·95	7·50	6·20
N/800	3·60	2·50	4·95	3·60
N/900	2·50	2·50	3·60	2·50

The differences in the values of osmotic pressure in roots when supplied with nitrogen in the form of ammonium sulphate and potassium nitrate are evident. In

the ammonium sulphate solution the osmotic pressure rises from 3·6 atms. to 6·2 atms. from N/100 to N/300, while the value of osmotic pressure remains steady in N/100 to N/300 potassium nitrate solutions. In the lowest concentration of the solutions of each, the values of osmotic pressure are the same. In the root base the osmotic pressure rises from 4·95 atms. to 8·8 atms. in N/100 to N/300 ammonium sulphate solutions, while the values of the osmotic pressure in the corresponding solutions of potassium nitrate are 2·5 atms. to 3·6 atms. The values of the osmotic pressure in the lowest concentrations of both are the same.

In the leaf sheath the osmotic pressure rises from 6·2 atms. to 11·5 atms. from N/100 to N/300 ammonium sulphate solutions while it is 3·6 atms. in the corresponding solutions of potassium nitrate. The osmotic pressure at the end of the series is 2·5 atms. in both.

At the leaf apex the osmotic pressure rises from 11·1 atms. to 15·9 atms., while it is 4·95 atms. in N/100 to N/300 solutions of each. In the lowest strengths of both the osmotic pressure is 3·6 atms. in $(NH_4)_2SO_4$ and 2·5 atms. in KNO_3 solutions.

The following table gives the values of suction pressure in the solutions of the two salts in different concentrations in the roots and leaves of fifteen days old seedlings.

TABLE XIX.

The comparison of suction pressures of the roots of seedlings grown in $(NH_4)_2SO_4$ and KNO_3 solutions.

Strength of the solution	Root apex		Root base	
	S. P. with $(NH_4)_2SO_4$ in atms.	S. P. with KNO_3 in atms.	S. P. with $(NH_4)_2SO_4$ in atms.	S. P. with KNO_3 in atms.
N/100	1·3	1·0	2·50	1·3
N/200	3·6	1·0	4·95	1·0
N/300	3·6	1·0	4·95	2·5
N/400	3·6	1·0	4·95	1·0
N/500	1·3	1·0	2·50	1·0
N/600	3·2	1·3	3·60	2·5
N/700	2·5	1·3	3·60	3·6
N/800	1·3	1·3	1·30	2·0
N/900	1·3	1·3	1·30	2·0

TABLE XX.

The comparison of suction pressures of the leaves of seedlings grown in $(NH_4)_2SO_4$ and KNO_3 solutions.

Strength of the solution	Leaf sheath		Leaf apex	
	S. P. with $(NH_4)_2SO_4$ in atms.	S. P. with KNO_3 in atms.	S. P. with $(NH_4)_2SO_4$ in atms.	S. P. with KNO_3 in atms.
N/100	3·60	2·00	7·50	3·20
N/200	7·50	1·30	8·80	2·50
N/300	8·80	2·50	13·00	4·40
N/400	7·00	2·50	8·80	4·40
N/500	3·60	2·50	6·20	4·40
N/600	4·95	2·50	6·20	4·40
N/700	3·60	3·60	5·65	5·65
N/800	2·50	2·00	3·60	2·50
N/900	2·00	2·50	3·20	3·10

The same type of differences in the values of suction pressure of roots and leaves in the corresponding solutions of ammonium sulphate and potassium nitrate as are observed in the values of osmotic pressure are noticed.

The chemical analysis of the different solutions of ammonium sulphate and potassium nitrate shows that the absorption of ions is closely related to the osmotic and suction pressures of the roots and leaves. The greater the absorption of a salt, the greater is the rise in the two pressures in the plants. The absorption of ammonium and sulphate ions decreases, as the concentration of the solution decreases from N/200 to N/900. The decreased absorption is accompanied by a decrease in the osmotic pressure and suction pressure of the roots and leaves.

TABLE XXI.

The quantities of NH_4'' and SO_4'' ions absorbed from different solutions of $(NH_4)_2SO_4$ and the suction pressure of the roots of seedlings grown in them.*

Strength of the solution	Amount of NH_4'' absorbed in grms.	Amount of SO_4'' absorbed in grms.	S. P. at the root base in atms.	S. P. at the root apex in atms.
N/200	0·0503	0·0191	4·95	3·60
N/300	0·0446	0·0180	4·95	3·60
N/400	0·0244	0·0138	4·95	3·60
N/500	0·0160	0·0116	2·50	1·30
N/600	0·0115	0·0090	3·60	3·20
N/700	0·0092	0·0088	3·60	2·50
N/800	0·0055	0·0085	1·30	1·30
N/900	0·0045	0·0084	1·30	1·30

* Analyses were made by Mr. T. J. Malkani.

Similarly potassium nitrate is very little absorbed during the early stages and very little change is noticed in the osmotic and suction pressures of plants kept in different concentrations of the potassium nitrate solution.

TABLE XXII.

The quantities of $K^{\cdot}$ and NO_3' ions absorbed from different solutions of KNO_3 and the suction pressures of the roots of seedlings grown in them.*

Strength of the solution	Amount of $K^{\cdot}$ absorbed in grms.	Amount of NO_3' absorbed in grms.	S. P. at the root base in atms.	S. P. at the root apex in atms.
N/1000	0·0075	0·0047	2·0	1·30
N/1100	0·0074	0·0045	2·5	1·00
N/1200	0·0074	0·0043	2·5	1·30
N/1300	0·0071	0·0040	2·0	1·30
N/1400	0·0066	0·0039	2·0	1·30
N/1500	0·0065	0·0036	2·5	1·30

As the quantities of different ions absorbed from the solutions of KNO_3 of different strengths are nearly the same, there are no wide differences in the suction pressures observed.

Similarly it is definitely proved by Mr. Malkani, that NO_3' ions are not absorbed by the rice seedlings in the early stages of growth from solutions of potassium nitrate of higher concentrations. These results will be published later in a separate paper.

The effect on the suction and osmotic pressures of the roots and leaves of the rice seedlings is determined, so far, by water culture experiments. It would be of interest to investigate if the same effects are produced on the osmotic and suction pressures of the rice seedlings grown in pots, even though the conditions in the soil are more complicated than those which are met with in water cultures. It was undertaken to see if a similar rise in the two pressures of the plant organs is noticed, when the rice seedlings are treated with solutions of ammonium sulphate. It was necessary to take uniform samples of soil in a set of pots to avoid complications arising out of the differences in the manurial constituents of the soil. Rice seedlings were raised in pots and on the seventh day the osmotic and suction pressures of the seedlings were measured. Then two pots were manured with N/200 ammonium sulphate solution. One pot was kept unmanured, and was watered with distilled water. Every subsequent day, all the pots were watered with distilled water. The osmotic and suction pressures of the roots and of leaves of the seedlings of manured and unmanured pots were measured on the 8th,

* Analyses were made by Mr. T. J. Malkani.

9th and 10th day after sowing. The following tables give the results of these determinations.

TABLE XXIII.

The osmotic pressure of the roots and leaves of rice seedlings unmanured and manured with N/200 $(NH_4)_2SO_4$.

Day	Root apex		Root base	
	Unmanured in atms.	Manured in atms.	Unmanured in atms.	Manured in atms.
8th day	3·6	4·4	4·4	5·5
9th day	2·5	4·4	3·6	5·0
10th day	2·5	4·4	3·6	5·0
	Leaf base		Leaf apex	
8th day	6·2	6·2	6·2	6·2
9th day	5·4	7·0	6·2	7·5
10th day	5·4	7·5	6·2	7·5

TABLE XXIV.

The suction pressure of the roots and leaves of rice seedlings unmanured and manured with N/200 $(NH_4)_2SO_4$.

Day	Root apex		Root base	
	Unmanured in atms.	Manured in atms.	Unmanured in atms.	Manured in atms.
8th day	2·0	2·5	2·5	3·6
9th day	2·0	3·1	2·5	4·4
10th day	2·0	3·1	2·5	4·4
	Leaf base		Leaf apex	
8th day	3·0	5·0	3·6	5·0
9th day	3·6	6·2	5·0	6·2
10th day	3·6	6·2	5·0	6·2

The differences in the osmotic and suction pressures of the roots and leaves of the unmanured and manured seedlings are obvious.

Similarly the rice seedlings were manured with N/300 ammonium sulphate and osmotic and suction pressures measured.

TABLE XXV.

The osmotic pressure of the roots and leaves of rice seedlings unmanured and manured with N/300 $(NH_4)_2SO_4$.

Day	Root apex		Root base	
	Unmanured in atms.	Manured in atms.	Unmanured in atms.	Manured in atms.
8th day	3·1	3·1	3·6	3·6
9th day	2·2	5·0	2·5	5·0
10th day	2·5	5·0	3·6	5·0
	Leaf base		Leaf apex	
8th day	3·6	3·6	5·0	5·0
9th day	2·5	5·6	3·6	7·5
10th day	3·6	6·2	5·0	7·0

TABLE XXVI.

The suction pressure of the roots and leaves of rice seedlings unmanured and manured with N/300 $(NH_4)_2 SO_4$.

Day	Root apex		Root base	
	Unmanured in atms.	Manured in atms.	Unmanured in atms.	Manured in atms.
8th day	2·0	2·0	2·5	2·5
9th day	2·0	3·6	3·1	4·4
10th day	2·0	3·1	2·5	3·6
	Leaf base		Leaf apex	
8th day	3·1	2·5	3·6	3·6
9th day	3·6	5·0	5·0	5·6
10th day	3·1	5·0	3·6	5·6

The results clearly indicate the rise in osmotic and suction pressures of the rice seedlings manured with ammonium sulphate.

Similar experiments with KNO_3 manuring could not be tried as the soil would very probably contain some nitrates and it would not be possible to compare the osmotic and suction pressures of the unmanured and manured plants; therefore, it was thought necessary to repeat these experiments with rice seedlings raised in sand. Again, the rise in osmotic pressure in soil may be also due to other changes, such as exchange of bases, or changes in the hydrogen-ion concentration of the soil when treated with ammonium sulphate. So attempts were made to grow seedlings in pure quartz sand in pots but they proved unsuccessful, very probably due to the quartz sand becoming a very compact mass, leaving not enough interspaces for air. So ordinary sea-shore sand was taken and digested with sulphuric acid and boiled for several hours. The sand was then repeatedly washed with distilled water and washings tested for various salts like chlorides, sulphates, phosphates and nitrates. The purified sand was taken for experiments when the filtrate did not give any reaction for any of the salts. The seedlings were raised in pots containing purified sand and on the 7th day one pot was manured with N/200 $(NH_4)_2SO_4$ solution, one with N/200 KNO_3 solution and one was kept unmanured. The osmotic and suction pressures of the leaves were then measured on the 9th day, 10th day and 13th day. The following tables give the results of these experiments.

TABLE XXVII.

The osmotic and suction pressures of the roots and leaves of unmanured and manured rice seedlings raised in pure sand.

Day	Leaf base			Root base		
	Unmanured	Manured with KNO_3	Manured with $(NH_4)_2SO_4$	Unmanured	Manured with KNO_3	Manured with $(NH_4)_2SO_4$
	Osmotic pressure in atms.					
9th . .	3·11	3·6	5·0	2·5	2·5	4·4
10th . .	3·6	3·6	5·0	2·5	3·1	4·4
13th . .	4·0	4·4	6·0	3·3	3·6	5·0
	Suction pressure in atms.					
9th . .	2·0	2·5	2·5	1·3	2·0	2·0
10th . .	2·0	2·5	3·6	1·3	2·0	3·6
13th . .	2·0	2·5	4·5	1·3	1·3	3·6

The differences in the osmotic and suction pressures of the roots and leaves of unmanured seedlings and seedlings manured with KNO_3 are very slight. There is an increase of about 0·5 atms. in the two pressures in seedlings manured with KNO_3, while the differences in osmotic and suction pressures of the rice seedlings manured with $(NH_4)_2SO_4$ and unmanured seedlings are quite appreciable.

It was then undertaken to study the effect of calcium and potassium on the osmotic and suction pressures of the rice seedlings, as it is known from the investigations of the earlier workers that these elements are absorbed by the rice plant. The solutions of calcium and potassium sulphates of different concentrations were prepared and the rice seedlings grown in beds of saw-dust were kept in these solutions. The osmotic and suction pressures of the roots and leaves were measured before putting them in these solutions. The seedlings were kept in these solutions for 6 to 7 days and the osmotic and suction pressures were determined.

TABLE XXVIII.

The osmotic and suction pressures of the rice seedlings in solutions of sulphates of calcium and potassium.

Salt	O. V. of the solution in atms.	O. P. at the root base in atms.	O. P. at the leaf apex in atms.	S. P. at the root base in atms.	S. P. at the leaf apex in atms.
		Date 4th July to 11th July 1930			
		Seedlings from garden soil			
$CaSO_4$ . . .	0·020	5·12	10·35	1·7	6·35
K_2SO_4 . .	0·020	3·80	8·98	1·4	5·18
		Date 11th July to 18th July 1930			
		Seedlings from pots			
$CaSO_4$ . . .	0·030	5·02	7·65	1·7	5·78
K_2SO_4 . .	0·030	3·80	6·60	1·5	4·20
		Date 8th July to 24th July 1930			
		Transplanted seedlings from the beds			
$CaSO_4$ . . .	0·020	6·35	14·70	3·8	10·35
K_2SO_4 . . .	0·020	4·20	12·96	2·49	9·98
$CaSO_4$ . . .	0·010	5·78	13·65	4·20	10·90
K_2SO_4 . .	0·010	4·20	12·96	3·20	8·98

It is evident from the results that the osmotic and suction pressures in the roots and leaves of the rice seedlings of different stages of growth are higher in the solutions of calcium sulphate than in the solutions of potassium sulphate. It was also determined by chemical analysis that calcium sulphate was absorbed in greater quantity than potassium sulphate.

It was undertaken to study the effect on the osmotic and suction pressures of the rice plants of culture solutions containing all the necessary elements and also of culture solutions in which one of the elements is omitted. The culture solution of the following composition was used:—KH_2PO_4 0·25 gm., $MgSO_4$ 0·5 gm., $CaSO_4$ 0·5 gm., KNO_3 1 gm., $FeCl_3$ 2 c. c. of 5 per cent. solution, water 10 litres.

The rice plants from beds were transferred to the solutions and the osmotic and suction pressures of the roots and leaves were measured after one week. The osmotic and suction pressures of the roots and leaves were recorded before keeping them in the culture jars.

TABLE XXIX.

The osmotic and suction pressures of the rice plants in culture solutions.

Date	O. P. at the root base in atms.	O. P. at the leaf apex in atms.	S. P. at the root base in atms.	S. P. at the leaf apex in atms.
14th August 1930—Before putting the plants in culture sol.	7·65	15·75	4·2	10·9
21st August 1930—After putting (I) the plants in culture sol.	8·98	15·75	5·02	9·3
21st August 1930—After putting (II) the plants in culture sol.	8·98	15·75	5·02	10·35
21st August 1930—Plants in the soil after a week.	8·98	15·75	5·02	10·9

The osmotic and suction pressures don't show any change in the culture solutions.

Culture solutions were also prepared in which potassium nitrate was replaced by ammonium sulphate and also culture solutions in which nitrogen was totally omitted.

TABLE XXX.

The osmotic and suction pressures of the rice plants in different culture solutions.

Date	O. P. at the root base in atms.	O. P. at the leaf apex in atms.	S. P. at the root base in atms.	S. P. at the leaf apex in atms.
Before putting the plants in culture sol.	7·65	15·75	4·2	10·9
20th August 1930—In culture sol. with $(NH_4)_2SO_4$ in place of KNO_3 for one week.	10·35	17·225	5·78	10·9
22nd August 1930—In culture sol. with $(NH_4)_2SO_4$ in place of KNO_3 for 9 days.	9·3	18·5	6·35	12·96
22nd August 1930—In culture sol. with no nitrogen for one week.	8·98	16·6	4·20	10·9
29th August 1930—In culture sol. without calcium.	8·98	16·6	5·02	10·9
29th August 1930—In culture sol. without iron.	8·22	15·75	3·8	10·9

It could be seen that omission of calcium, nitrogen and iron has a visible effect on the osmotic and suction pressures of roots and leaves. The nitrogen in the form of ammonium sulphate raises the osmotic and suction pressures of the rice plants in comparison to the pressures in the plant organs in the normal culture solution containing nitrate nitrogen.

All these results described above support the idea that the absorption of the ions increases the osmotic and suction pressures of plants and the omission of the useful ions from the culture solutions results in small depressions of the osmotic and suction pressures of the roots and leaves.

The abovementioned experiments on the rice plant clearly show the effect on the suction and osmotic pressures of the roots and leaves of ions which are absorbed by the plants. The effect of ammonium sulphate is clearly visible, as there is a rise in the two pressures. The same is true of other necessary ions absorbed by the rice plant.

It was undertaken to find out if the osmotic and suction pressures of the roots and leaves of other plants were similarly affected by the absorption of ions. If the osmotic and suction pressures rise on account of the absorption of some ions of a salt and no such rise of the two pressures is observed when plants are treated with other salts, it would be possible to determine the salts absorbed by the plants by this method, instead of the current method of making the chemical analysis of the plant.

These experiments could not be carried out by ordinary pot culture method, as the soil may contain all the necessary elements in adequate quantities and the addition of a salt that is absorbed by a plant may not cause a rise in the osmotic and suction pressures.

In performing these experiments by water culture methods, there are difficulties. When plants are treated with a solution of any one salt the osmotic and suction pressures of the plant may show a fall as compared to the pressures existing in the plants in the soil or to the pressures in the plants in the culture solutions before it is transferred to a single salt solution even though the salt is absorbed. To overcome this difficulty, it is necessary to compare the osmotic and suction pressures of the roots or leaves of a plant in solutions of two or three salts used singly at a time. The values of osmotic and suction pressures will be higher in the solutions of that salt which is more absorbed than the other two.

Tradescantia zebrina, Hort. is selected for the experiments as it is a plant which can be readily grown in culture solutions. The suction pressure of the leaves was determined before the plants were transferred to the culture solutions. The suction pressure of the leaves was then determined at short intervals after it was kept in the culture solutions. After the suction pressure of the leaves became steady the plants were kept in the solutions of potassium nitrate, potassium sulphate, ammonium

sulphate, ammonium nitrate and culture solution containing no potassium, and culture solution without potassium and nitrogen together. The osmotic value of each salt solution was calculated before the plants were kept in them.

TABLE XXXI.

The suction pressure in the leaves of Tradescantia zebrina, *Hort. in different solutions.*

Solution	O. V. of the solution in atms.	S. P. before transfer in atms.	S. P. after 4 days in atms.	S. P. after 21 days in atms.	S. P. after 28 days in atms.	S. P. after 37 days in atms.	S. P. after 50 days in atms.
Culture . . .	0·0546	2·4	3·05	3·05	3·05	2·40	2·40
N/100 KNO_3 . .	0·376	2·4	3·05	3·05	3·05	2·40	2·40
N/200 KNO_3 . .	0·188	2·4	2·40	3·05	3·05	2·40	2·40
N/100 K_2SO_4 . .	0·516	2·4	2·40	1·85	1·85	1·85	1·85
N/200 K_2SO_4 . .	0·258	2·4	1·85	1·85	1·85	1·10	1·10
N/200 $(NH_4)_2SO_4$.	0·264	2·4	2·70	2·40	2·40	2·40	2·40
N/200 NH_4NO_3 . .	0·148	2·4	1·85	1·10	1·10	1·10	1·10
Culture without K .	0·057	2·4	1·85	1·85	1·10	..	..
Culture without K & N .	0·0171	2·4	1·85	1·10	1·10	..	..
Culture . . .	0·0546	2·4	3·05	3·05	3·05	2·40	2·40

In ordinary culture solution the suction pressure in the leaves rises to 3·05 atms. after a few days and it remains the same for 28 days, after which the suction pressure falls to its original value. If the plants are kept in fresh culture solutions, the suction pressure shows the same rise and fall. When the plants are transferred to KNO_3 solution of N/100 concentration, the suction pressure again rises to 3·05 atms. after a few days and shows the same changes as in culture solution. In N/200 KNO_3 the rise in suction pressure is not observed on the fourth day, but is seen later. In N/100 K_2SO_4 solution the suction pressure goes down to 1·85 atms. and in N/200 K_2SO_4 it goes down to 1·1 atms. In ammonium sulphate solution the suction pressure suffers no change, while in ammonium nitrate it goes down to 1·1 atms. The effect of omission of potassium and potassium and nitrogen together from the culture solution is quite apparent by the sudden fall in the suction pressure.

The rise and fall in the suction pressure of the leaves are, so far as the above results show, independent of the osmotic values of the solution outside. Though the osmotic value of N/100 KNO_3 solution is about seven times greater than the osmotic value of the culture solution, the changes in the suction pressure of leaves are the

same in both cases. The osmotic values of N/200 K_2SO_4 and N/200 $(NH_4)_2SO_4$ are the same, but the suction pressure of the leaves of the plants in the two solutions are different.

The above conclusions hold good when the two external solutions containing *different* salts of the same osmotic values are taken for comparison. They do not hold good for solutions of the same salt like N/100 and N/200 K_2SO_4 for the differences in the concentration of the same salt will have different effects on the suction pressures of the plant.

The suction pressures of the leaves rises in solutions containing nitrates except ammonium nitrate, but the rise remains constant for about 28 days after which there is a fall. There is a fall in the suction pressure in the solutions in which nitrate ion is absent. Similarly the potassium ion is absorbed by the plant, as the suction pressure falls in culture solution from which potassium is omitted. It is evident from the results that potassium and nitrogen are absorbed as the suction pressure of the leaves shows a definite rise for a short period. In the case of other salts there is a gradual fall in the suction pressure, which is probably caused by absorption of water which is not accompanied by absorption of a salt in solution in adequate quantities.

Similar experiments were tried with the maize seedlings. The maize seedlings about fifteen days old were transferred to culture solution and later to solutions of ammonium sulphate, potassium sulphate and to culture solution containing ammonium sulphate in place of potassium nitrate. The measurements of the suction pressure of the roots were made at short intervals.

TABLE XXXII.

The suction pressure of the roots of Zea Mays *L. in different solutions.*

Solution	O. V. of the sol. in atms.	S. P. of root before transfer in atms.	S. P. after 5 hrs. in atms.	S. P. after 24 hrs. in atms.	S. P. after 4 days in atms.	S. P. after 7 days in atms.	S. P. after 10 days in atms.	S. P. after 14 days in atms.
Culture with KNO_3 .	0·0546	1·97	1·97	1·97	2·2	2·40	2·00	1·85
Culture with $(NH_4)_2SO_4$	0·0570	1·97	1·97	1·97	1·85	1·85	..	1·85
Culture with $(NH_4)NO_3$	0·0540	1·97	1·97	1·85	1·42	..	..	..
N/100 KNO_3 . .	0·3760	1·97	1·97	1·97	2·40	2·40	2·20	1·85
N/100 $(NH_4)_2SO_4$.	0·5280	1·97	1·97	1·97	1·61	1·61	1·85	1·85
N/100 K_2SO_4 . .	0·5160	1·97	1·97	1·42	1·10	1·10	1·20	1·20

The suction pressure of the roots rises, on the 4th day in culture solution containing KNO_3 and in N/100 KNO_3 solution. The rise is not maintained for a long time, as the suction pressure falls to 1·86 atms. on the 14th day. In other salt solutions the suction pressure of the roots has fallen. The results show that KNO_3 raises the suction pressure of the roots for a short period and this rise in suction pressure indicates that the potassium is needed and absorbed by the maize seedlings which are also well known facts.

It was then undertaken to see the effect of nitrates other than KNO_3 on the suction pressures of the roots and leaves of the maize seedlings. The maize plants were kept in solutions of calcium nitrate and magnesium nitrate and the suction pressures of the roots and leaves were measured at short intervals. Side by side the effect of omission of KNO_3 (*i.e.*, without K and N) from normal culture solution was also studied. The following table gives the results of the suction pressure of the roots of the maize plants.

TABLE XXXIII.

The suction pressure of the roots of the maize.

Salt	O. V. of solution in atms.	S. P. before in atms.	S. P. after 1 day in atms.	S. P. after 4 days in atms.	S. P. after 9 days in atms.
N/200 $Ca(NO_3)_2$ . . .	0·214	2·2	2·2	1·42	Roots died
N/500 $Ca(NO_3)_2$ . . .	0·089	2·2	2·2	1·42	,, ,,
N/1000 $Ca(NO_3)_2$ . . .	0·043	2·2	2·2	1·85	,, ,,
N/200 $Mg(NO_3)_2$ . . .	0·192	2·2	2·2	1·42	,, ,,
N/500 $Mg(NO_3)_2$ . . .	0·077	2·2	2·2	2·40	1·85
N/1000 $Mg(NO_3)_2$ . . .	0·038	2·2	2·2	2·70	2·50
Culture	0·055	2·2	2·2	2·70	2·70
Culture without K & N . .	0·017	2·2	2·2	1·85	Roots died

The suction pressure falls on the 4th day in $Ca(NO_3)_2$ solutions of all strengths. It appears from the results that the ions are absorbed from N/1000 $Ca(NO_3)_2$, N/500 and N/1000 $Mg(NO_3)_2$, as the suction pressure of the roots is higher in these dilute solutions than it is in stronger ones. In N/500 and N/1000 $Mg(NO_3)_2$ the suction pressure of the roots has slightly risen.

In normal culture solution a rise in the suction pressure is again observed, while in the culture solution devoid of potassium and nitrogen, there is a distinct fall in the suction pressure of the roots.

The suction pressure of the leaves of the maize plant shows similar rise and fall as in the roots.

TABLE XXXIV.

The suction pressure of the leaves of the maize plant.

Salt	O. V. of solution in atms.	S. P. before in atms.	S. P. after 1 day in atms.	S. P. after 4 days in atms.	S. P. after 9 days in atms.
N/200 $Ca(NO_3)_2$ . . .	0·214	3·05	3·05	2·70	Leaves died
N/500 $Ca(NO_3)_2$ . . .	0·089	3·05	3·05	2·70	,, ,,
N/1000 $Ca(NO_3)_2$ . . .	0·043	3·05	3·05	2·90	,, ,,
N/200 $Mg(NO_3)_2$ . . .	0·192	3·05	3·05	2·70	,, ,,
N/500 $Mg(NO_3)_2$ . . .	0·077	3·05	3·05	3·05	2·90
N/1000 $Mg(NO_3)_2$ . . .	0·039	3·05	3·05	3·05	3·05
Culture	0·055	3·05	3·05	3·20	3·20
Culture without K and N .	0·017	3·05	3·05	2·70	Leaves died

Here also there is a clear indication of a rise in suction pressure in normal culture solution, while there is a fall in the suction pressure of the leaves in all $Ca(NO_3)_2$ solutions, in N/200 $Mg(NO_3)_2$ solution and in culture solution devoid of potassium and nitrogen. In N/500 and N/1000 magnesium nitrate solutions the suction pressure remains the same on the fourth day.

CONCLUSIONS.

The osmotic pressure and suction pressure of the roots and leaves of the rice seedlings are higher than the osmotic values of the surrounding solutions. These results are not in accord with those obtained by Ursprung and Blum [1921] with *Vicia faba*, L., where they find that the suction pressure of the roots becomes equal to the osmotic pressure of the external solutions. The divergence of the results obtained here could easily be explained. Ursprung and Blum [1921] have used cane sugar solutions as an external medium in which the plants are kept. As cane sugar is not permeable to the roots, the exchange of water only takes place between the external medium and the cells of the roots and the suction pressure of the roots becomes equal to the osmotic pressure of the solution, so that no more exchange of water in any direction occurs. This is in accordance with the physical phenomenon of osmosis. But in the case of solutions containing salts which are absorbed by the roots, the suction pressure of the roots is also affected by the endosmosis of the

ions. In ammonium sulphate solutions the osmotic and suction pressures of the roots and leaves were much higher than the osmotic values of the ammonium sulphate solutions (Tables I to VIII). In potassium nitrate solutions the osmotic and suction pressures of the roots and leaves did not show any rise in 8 days and would have fallen, if the seedlings were kept for a long time, which was not possible as the seedlings did not keep healthy in these solutions for a longer period. (Tables IX to XVI).

The results clearly show that the rise in the suction and osmotic pressures of the leaves and roots in ammonium sulphate solutions is due to the absorption of ammonium and sulphate ions as determined by the chemical analysis of the ammonium sulphate solutions after the seedlings had remained in them for eight days (Table XXI). The rise is greatest in N/300 $(NH_4)_2SO_4$ solution. In KNO_3 solution there is no such rise observed in the osmotic and suction pressures of the roots and leaves, as it is found that very little of nitrate ion is absorbed by the rice seedlings (Table XXII).

The experiments with single salt solutions of different osmotic values of calcium sulphate and potassium sulphate show that the suction and osmotic pressures of the leaves and roots of the rice plants are higher in calcium sulphate solutions than in the solutions of potassium sulphate. It is probable that calcium sulphate is absorbed more than the potassium sulphate during the early stages of growth before and just after transplantation (Table XXVIII).

The suction and osmotic pressures of the leaves and roots of the rice plant in the soil are found to be the same as in normal culture solution. However, when the rice plants are kept in culture solution containing ammonium sulphate, the suction and osmotic pressures of the roots and leaves are higher than the suction and osmotic pressures of roots and leaves of plants in the normal culture solution (*i.e.*, with nitrate nitrogen). The osmotic pressure of the leaves rose in 9 days from 12·96 atms. to 18·5 atms. and the suction pressure went up to 12·96 atms. from 10·9 atms. The rise is due to the plant absorbing ammoniacal nitrogen more than nitrate nitrogen. The omission of nitrogen or calcium or iron from culture solutions are accompanied by the absence of any rise in the two pressures (Table XXX).

Experiments with other plants, performed to obtain support for the view that absorption of a salt is indicated by a rise in the suction and osmotic pressures of a plant, gave very confirmatory results. The plants selected were *Tradescantia zebrina* Hort. and *Zea Mays* L. as both are known to flourish in culture solutions. The suction pressure of the leaves of *Tradescantia zebrina* Hort. shows a rise in four days in the normal culture solution (with potassium nitrate) and also in pure potassium nitrate solutions, remains constant for 3 or 4 weeks and then begins to fall (Table XXXI). The suction pressure of the leaves, on the contrary, begins to fall on the 4th day in the single salt solutions of potassium sulphate, ammonium sul-

phate, ammonium nitrate and in the culture solutions from which either potassium or potassium and nitrogen together are omitted (Tables XXXI and XXXII). The results show the importance of potassium nitrate for the growth of the plant as it is absorbed more than other salts, as can be seen safely from the rise in the suction pressure. Similar results are obtained with maize seedlings. The suction pressure of the roots and leaves shows a rise in solutions of potassium nitrate and in culture solutions containing potassium nitrate (Tables XXVIII to XXXIV).

A careful study of the results shows that the rise or fall in suction pressure of a plant is independent of the osmotic value of the external solution. This conclusion is opposite to that drawn by Ursprung and Blum [1921]. The osmotic values of the two solutions may be the same but the suction pressure of the plants in the two solutions may be quite different. This may be due to the difference in the amounts of the two salts absorbed from the solutions. The results of Ursprung and Blum [1916, 1, 2 and 3] have been explained above.

To summarize briefly the results of about seven hundred and fifty determinations in this investigation, the changes in the osmotic and suction pressures of a plant organ like the root or the leaf may give some indication of the salts absorbed by the plants, and consequently of the salt requirements of the plant, unless it be that the salt absorbed is not required by it. In the opinion of the authors, this method of determining salt requirements of a plant can be employed in preference to the lengthy method of chemical analysis of a plant at different stages of growth. It can be safely concluded that by this method the most important of the salts absorbed by the plants can be determined by noticing the changes in the osmotic or suction pressures of the roots or the leaves of a plant.

Summary.

(1) In all the previous investigations dealing with the salt requirements of the rice plant, no attempt is made to determine the changes produced in the osmotic and suction pressures of the plants kept either in culture solutions of different compositions or when treated with certain fertilizers in the soil. It was therefore considered necessary to investigate the changes produced in the osmotic and suction pressures of the rice plant under different treatments. It would also be of interest to study the changes in the two pressures of the plant when it is treated with salts which are either not absorbed in appreciable quantities during the life cycle or at certain stages of growth of the plant.

(2) If the results of the investigation would show that there is a rise in the osmotic and suction pressures of the rice plant when treated with salts which are absorbed and if no such rise is noticed when the rice plant is treated with salts

which are not absorbed, it would be worth while to investigate if this method can be developed to determine the salt requirements of plants.

(3) The effect on the osmotic and suction pressures of roots and leaves of the rice seedlings (Kolamba variety No. 42) of different stages of growth when treated with solutions of ammonium sulphate of concentrations varying from N/100 to N/900 is studied. Similarly the effect on the osmotic and suction pressures of the roots and leaves of the rice seedlings when kept in solutions of KNO_3 of concentrations varying from N/100 to N/1500 is studied. The results of these experiments show that the osmotic and suction pressures of the roots and leaves of the rice seedlings in ammonium sulphate solutions are generally higher than those of the same organs of the rice seedlings in KNO_3 solutions, except in solutions of $(NH_4)_2SO_4$ of lowest strengths. There is also a big rise in the two pressures after the seedlings are transferred to $(NH_4)_2SO_4$ of concentrations varying from N/100 to N/500, while no such rise is noticed in the corresponding solutions of KNO_3.

(4) The chemical analysis of the solutions, after the seedlings had remained in them for 8 days, shows that $NH_4^{\cdot}$ and SO_4'' ions are considerably absorbed, varying according to the concentrations of $(NH_4)_2SO_4$ solutions, while NO_3' ions are absorbed in minute quantities from different solutions of KNO_3.

(5) Similar pot culture experiments both in garden soil and in pure sand show that there is a rise in the osmotic and suction pressures of the rice seedlings manured with $(NH_4)_2SO_4$ solutions as compared with the unmanured seedlings, while very little rise is observed in the two pressures of the seedlings when manured with KNO_3.

(6) The changes in the osmotic and suction pressures of the roots and leaves of the rice plant in single salt solutions of calcium sulphate and potassium sulphate are studied. The results show that the pressures are higher in calcium sulphate solutions than in potassium sulphate solutions.

(7) The effect on the osmotic and suction pressures of the roots and leaves of the rice plants when treated with ammonium sulphate and potassium nitrate solutions in later stages of growth is studied with similar results as mentioned above.

(8) The osmotic and suction pressures of the roots and leaves of the rice seedlings in normal culture solutions are first recorded. The changes in the two pressures are then studied in culture solutions from which potassium or potassium and nitrogen, or ammonium, or calcium is omitted. Distinct differences in the two pressures are observed.

(9) Similar experiments with the roots and leaves of *Tradescantia zebrina* Hort. and *Zea Mays* L. are performed to see if any rise or fall in the osmotic and suction pressures is observed when a salt that is required by the plants is supplied or omitted. The results show a rise when the salt that is needed is added and a depression when it is omitted.

(10) The suggestion is put forward as a result of seven hundred and fifty determinations of the osmotic and suction pressures that the most essential of the salts required by plants can be determined by changes in the osmotic and suction pressures of their roots and leaves.

REFERENCES.

Adie, R. H. (1891). *J. Chem. Soc.* 352.

Copeland, E. B. (1924). Rice. Macmillan and Co., Ltd.

Dastur, R. H. and Ella Baptista. (1931) *Ind. J. of Agric. Sci.* **1**, 165-188.

Demidenko, T. (1926). *J. Landw. Wissensch. Moskau* **3**, 300-313 (quoted in Bot. Centralblatt. Neue Folge Band 9, Heft 1-2).

De Vries. (1884, 1). *Jahrb. f. Wiss. Bot.* **14**, 427-601 (quoted by W. Stiles, 1924).

——— (1884, 2). *Bot. Zeitung.* **42**, 289 (quoted by W. Stiles, 1924).

Espino, R. B. (1920). *Philippine J. of Sci.* **16**, 455-527.

Gericke, W. F. (1930). *Soil Sci.* **39**.

Gile, P. L. and Carrero, J. A. (1915). *J. Agric. Res.* **3**, 205-210.

Hofler, K. (1918). *Ber. deut. Bot. Ges.* **36**, 414-422 (quoted by W. Stiles, 1924).

Kellner, O. (1884). Agricultur Chemische Studien uber die Reiscultur Landw. Ver suchsst. **30**, 18-41 (quoted by Copeland, 1924).

Kelley, W. P. (1911). *Hawaii. Agric. Expt. Bull.* No. 24.

Kelley and Thompson (1910). *Hawaii. Agric. Expt. Sta. Bull.* No. 21.

Litwinow, L. (1926). *Bull. Inst. rech. biol. Univ. Perm.* **4**, 447-469 (quoted in Bot. Centralblatt. 1927 Neue Folge Band 9, Heft 5-6).

Molz, F. J. A. (1926). *Amer. J. Bot.* **13**, 433-464.

Morse, H. H. (1914). Carnegie Institute publication, Washington, **198** (quoted by W. Stiles, 1924).

Nagaoka, M. (1904). *Bull. Coll. Agric. Imp. Univ. Tokyo,* 285-334 (quoted by Copeland, 1924).

Sahasrabuddhe, D. L. (1928). *Dept. of Agric. Bombay, Bull.* No. 154.

Stiles, W. (1924). Permeability. New Phytologist Reprint No. 13. Wheldon and Wesley Ltd., London.

Trelease, S. (1920). *Philippine J. of Sci.* **16**, 603-630.

Ursprung and Blum (1916, 1). *Ber. deut. Bot. Ges.* **34**, 88-104 (quoted by W. Stiles, 1924).

Ursprung and Blum (1916, 2). *Ber. deut. Bot. Ges.* **34**, 525-539 (quoted by W. Stiles, 1924).

Ursprung and Blum (1916, 3). *Ber. deut. Bot. Ges.* **34**, 539-554 (quoted by W. Stiles ,1924)

Ursprung and Blum (1921). *Ber. deut. Bot. Ges.* **39**, 139-148.

Ursprung and Blum (1923). *Ber. deut. Bot. Ges.* **41**, 338-343.

STUDIES ON BACTERIOPHAGES OF THE ROOT NODULE ORGANISMS.

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(With Plates XIII—XV and one text-fig.)

In 1923, Gerretsen, Grijns *et al* first reported that root nodules of leguminous plants yielded a bacteriophage which brought about the dissolution of the organisms of the root nodules. They worked on bacteriophages derived from the root nodules of clover, lupin and serradella. The bacteriophages they isolated brought about the dissolution of bacteria rather slowly, requiring about ten days at ordinary temperatures. The bacteriophages were reported to be specific in their action on the bacteria of the species of leguminous plants from which they were isolated; the bacteriophage isolated from clover root nodules had no appreciable action on organisms from lupin or serradella and *vice versâ*.

After many unsuccessful attempts to confirm the results of the previous workers, Hitchner [1930] was able to isolate a bacteriolytic principle from red clover root nodules which was active only against one strain of the organisms isolated from red clover root nodules. In the course of his work numerous nodules taken at various ages from pea, bean, sweet clover, alfalfa, as well as from other red clover plants had been examined as to the presence of a lytic principle without success.

The lack of uniformity in the details in the procedure for the isolation of these bacteriophages has been a great handicap in obtaining reproducible results and in comparing the investigations of different workers in this field. By introducing some modifications in the procedure we have found a method which has given consistently reproducible results. The details of the method are given below:—

METHOD OF ISOLATION OF THE BACTERIOPHAGES.

Ten to twelve nodules from the roots of plants three to eight weeks old were treated with 0·1 per cent. $HgCl_2$ solution for 10 minutes. The sterilized nodules were repeatedly washed with sterile water and were then crushed with a sterile

glass rod in Marmite Mannite Broth. The Marmite Mannite Broth contained the following ingredients:—

Mannite . . .	2·0 gms.	Marmite	1·0 gm.
K_2HPO_4 . . .	0·5 gm.	$MgSO_4$	0·2 ,,
KNO_3 . . .	0·2 ,,	Water	1000 c.c.

The reaction of the medium was adjusted to pH 7·8 and each test tube containing 10 c.c. of the medium received 0·1 gm. of $CaCO_3$ before sterilization at 120°C. for 30 minutes. This medium has proved to be most suitable for the isolation of the bacteriophages in our experiments. A part of the emulsion of the crushed root nodules prepared as described above was plated for the isolation of pure strains of the nodule organisms. The remaining emulsion was incubated for 5 to 10 days and filtered successively through two porcelain filter candles. The filtering apparatus was assembled as follows (Fig. 1.):—

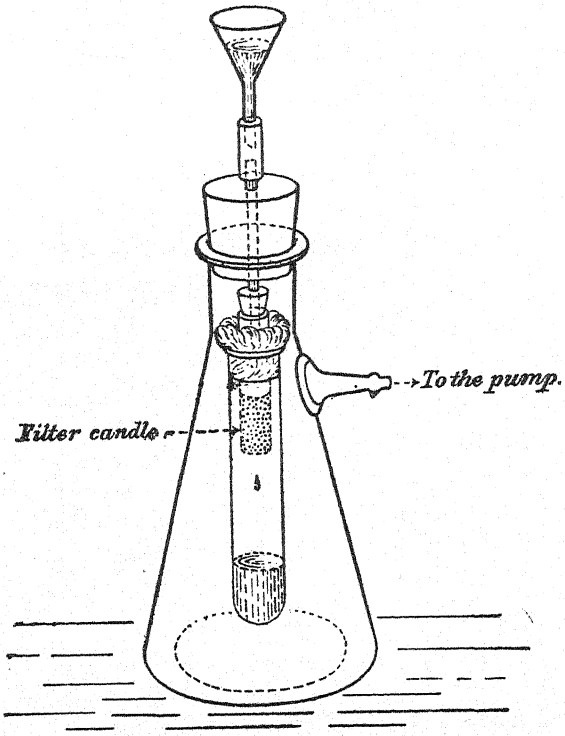


Fig. 1. Ultrafiltration apparatus fully assembled.

The Pasteur Chamberland filter candle was mounted in a test tube by rolling a narrow band of non-absorbent cotton about the glazed porcelain portion of the candle in such a manner that a plug was formed fitting firmly into the test tube. The apparatus was then sterilized by dry heat. For the filtration, the long stem

of a funnel was first inserted into a large rubbur stopper which fitted the neck of a filter flask, and then in another small rubber stopper below the larger one, the latter fitted tightly into the mouth of the sterilized filter candle. The fluid to be filtered was poured through a filter paper into the funnel and a moderate vacuum (20 c.c. M) established in the filter flask by a water pump. When the filtration was complete, the large stopper was removed, bringing with it the candle in the glass test tube. The filter candle with the cotton plug were removed and a sterile cotton plug was inserted after passing the mouth of the tube containing the filtrate through a gas flame, to prevent outside contamination. The filter candles and the funnel with the two corks were heated for 30 minutes in a large volume of distilled water immediately after being used for filtration. If the filter candles were allowed to dry before being boiled the pores were found to be blocked and filtration through such filter candles took a very long time. After boiling, the filter candles were dried in an oven at 100°C. and remounted as before for further use.

Filtrates were often found to give bacterial growth. This is presumed to be due to the fact that bacteroids that could pass through the filter candle were present in the nodules and specially in cultures with bacteriophages. Pasteur Chamberland filter candles of porosity ranging from L7 to L13 were tried with a view to excluding the organisms which found their way in the filtrate but in many cases the filtrate was not found to be absolutely free from the bacteroids of the nodule organisms. The age of the culture which was being filtered had a marked effect on the number of organisms passing through. As a general rule the number of organisms, found in the filtrate, diminished as the culture grew older. Though the filtrates were not sterile, outside contamination never occurred.

Two drops of the filtrate were added to one batch of tubes seeded with six strains of the corresponding nodule organisms immediately after inoculation; a second batch of seeded tubes was allowed to become cloudy and the same amount of the filtrate was then added on the following day, a third batch of seeded tubes was kept as controls. In the beginning the tubes receiving the filtrate did not show any clearing, but sometimes were more turbid than the control tubes. The former were filtered again after five days even when no clearing was apparent, and this second filtrate was again added to three series of tubes as in the first instance. The serial transfers were continued in this way till some sign of dissolution of bacteria was seen by a difference in cloudiness of tubes in the different batches. The first sign of the presence of the bacteriophages was observed in tubes receiving the filtrate simultaneously with the organisms. Twenty-four hours after the inoculation of the bacteriophage and the organisms, there was only slight flocculent growth as compared to the growth in the control tubes. Further clearing of the

cloudy tubes took place in 48 to 72 hours. The tubes in which the filtrate was added subsequent to one day's growth of the organisms cleared only when the bacteriophage was of sufficiently high virulence. The contents of completely clear or slightly turbid tubes were filtered at the end of five days. The serial transfers were then carried out decreasing the amount of filtrate added each time till one loopful (·01 c.c.) completely inhibited the growth when simultaneously inoculated with the organisms in 24 hours. At this stage the tubes receiving the filtrate subsequent to 24 hours' growth of the organisms showed more or less complete clearing. Sometimes it was observed that one or more strains out of the six selected were more easily dissolved than the others, in which case the virulence of the bacteriophage was enhanced at the expense of these strains.

Using the above technique the lytic principles from root nodules of various plants were isolated and a few studied. The initial virulences of the bacteriophages occurring in different plants for the corresponding organisms were different, since the number of serial transfers which were required to show distinctly a definite presence of the bacteriophage varied in different plants. The bacteriophage isolated from *Trifolium* root nodules showed its presence after two transfers while that from *Lathyrus* showed it after ten serial transfers.

The effect of basal medium on virulence.

D'Herelle [1926] has pointed out that while the interior of the bacterial cells is the true and sole medium for the multiplication of the bacteriophage, the medium in which the organisms are suspended also plays an important role. The importance of the selection of this basal medium for isolation as well as for the enhancement of virulence was neglected by previous workers. Hitchner [1930] failed to enhance the virulence of the bacteriolytic principle isolated by him beyond 1×10^{-5}. He suggested that the inability to enhance the virulence further was due to the probable loss in filtration or inherent resistance of the homologous strain and no attempt was made by him to enhance the virulence of the phage by change of medium. In our experiments also it was not possible to enhance the virulence of the bacteriophage beyond 1×10^{-4} by using the yeast infusion broth according to Hitchner's formula. An addition of 0·2 per cent. mannite to Hitchner's broth helped to enhance the virulence to 1×10^{-6} and after a few more passages in the yeast infusion broth with 0·5 per cent. mannite the virulence was found to have been increased to 1×10^{-8}. The experiments definitely showed that most probably Hitchner failed to enhance the virulence of the bacteriolytic principle because of the unsuitability of the medium used by him.

D'Herelle [1926] has pointed out that as a general rule the most favourable medium for the action of the bacteriophage to proceed most perfectly should have a

composition best suited to the development of the particular bacterial type selected to undergo dissolution. Joshi [1920] while comparing various media for the growth of nodule organisms, found that soil extract mannite was the best medium, for it gave better growth than soil extract maltose, synthetic agar or levulose agar and other sugar media employed by various previous workers. Among sugars mannite and sucrose seemed to give a consistently good growth of the organisms, while lactose gave the least growth. But the growth factor was not the only one to be considered while selecting a suitable medium for work on the bacteriophages of these organisms. The production of slime by the organisms was a very important factor to be considered, since this might make it difficult for the bacteriophage to attack the organisms ; a medium which retarded the production of slime by the organisms was therefore considered necessary. The addition of small amounts of KNO_3, $(NH_4)_2SO_4$ or other easily assimilable forms of available nitogen in the medium has been found to decrease slime production. The amount and kind of sugar in the medium also affects slime production as well as the change of the reaction of the medium.

In the early stages it was realised that the media suitable for isolation were unsuitable for the enhancement of virulence of the bacteriophage to a maximum point and also that the media used for the enhancement of virulence were unsuited for the isolation of the bacteriophage, especially if it was of a very weak virulence. It was necessary to use two media, *viz.*, one with no sugar, or only a very small amount for isolation, and for the enhancement of the virulence of the isolated bacteriophages, another medium containing 0·5 per cent. sugar. Small amounts of other constituents of the medium such as KNO_3 or $(NH_4)_2\ SO_4$, which helped in isolation were found adverse to the enhancement of virulence. A number of media were therefore made with varying composition to see the effect of different constituents on the bacteriophage and to find the composition of the medium that would give maximum enhancement of virulence. A statement showing the enhancement of virulence of the three different bacteriophages in different media after five successive passages is given in Table I.

TABLE I.

Virulence of bacteriophages attained after 5 passages.

Bacterio-phages	Initial virulence	Medium								
		A	B	C	D	E	F	G	H	I
Trifolium, alex.	1×10^{-5}	1×10^{-3}	1×10^{-5}	1×10^{-7}	1×10^{-6}	1×10^{-8}	1×10^{-6}	1×10^{-3}	1×10^{-7}	1×10^{-3}
Lathyrus, Odor.	1×10^{-5}	1×10^{-3}	1×10^{-5}	1×10^{-7}	1×10^{-6}	1×10^{-8}	1×10^{-6}	1×10^{-3}	1×10^{-7}	1×10^{-3}
Dolichos, Lablab.	1×10^{-7}	1×10^{-4}	1×10^{-7}	1×10^{-8}	1×10^{-6}	1×10^{-10}	1×10^{-6}	1×10^{-2}	1×10^{-9}	1×10^{-5}

The description of the media is given below :—

Medium	A	Medium	B=	Medium	A+0·2 per cent. mannite
Composition					
K_2HPO_4 .	0·5 gm.	,,	C=	,,	A+0·5 per cent. ,,
$MgSO_4$.	0·2 ,,	,,	D=	,,	A+1·0 per cent. ,,
KNO_3 or $(NH_4)_2SO_4$	0·2 ,,	,,	E=	,,	C—without KNO_3 or $(NH_4)_2SO_4$.
			F=	Marmite replaced by clover extract made by extracting 250 gms. clover plants with 500 c. c. of water. 100 c. c. of the extract used for 1 litre of the medium.	
Marmite .	1·0 ,,	,,	G=	Ashby's mannite broth.	
Water .	1000 cc	,,	H=	Medium E with mannite replaced by sucrose.	
...	...	,,	I=	Medium E with mannite replaced by lactose.	

To see what effect the reaction of the medium had on the action of the bacteriophage, medium E which gave the highest enhancement in the last experiment was chosen and the enhancement of virulence of the bacteriophage was observed by adjusting it to different hydrogen ion concentrations. The results are set forth in Table II.

TABLE II.

Virulences after two passages in medium E at different H· concentration.

Bacteriophages.	Initial virulence	pH 7·0	pH 7·4	pH 7·6	pH 7·8	pH 8·0	pH 8·2	pH 8·4	pH 8·6
Trifolium alexandrianum	1×10^{-8}	1×10^{-5}	1×10^{-6}	1×10^{-8}	1×10^{-8}	1×10^{-8}	1×10^{-8}	1×10^{-6}	1×10^{-4}
Lathyrus Odoratus .	1×10^{-8}	1×10^{-5}	1×10^{-6}	1×10^{-8}	1×10^{-8}	1×10^{-8}	1×10^{-8}	1×10^{-6}	1×10^{-4}
Dolichos, Lalbab .	1×10^{-10}	1×10^{-5}	1×10^{-7}	1×10^{-10}	1×10^{-9}	1×10^{-10}	1×10^{-10}	1×10^{-8}	1×10^{-7}

The results show that the reaction best suited for the action of the bacteriophage was between pH 7·6 and pH 8·2.

The temperature best suited for bacteriophagy was found to be 30°C. At the higher temperature of 37°C. the bacteriophaged cultures showed a speedy development of secondary cultures while at temperatures below 30°C. the time taken by the bacteriophage to dissolve the suspensions of the organisms was much greater than that at 30°C.

LYSIS ON THE SOLID MEDIA.

(*a*) *Plaque formation.*— In 1923 Gerretsen and Sohngen and Grijns reported independently of each other, that the bacteriophages exerting a lytic action against *B. Radicicola* occurred in the root nodules of Leguminoseae. They had, however, failed to obtain characteristic plaque formation which test only could establish that the lytic agent was a true bacteriophage, for there are some lytic agents, that inhibit the growth of organisms without being true bacteriophages. A few years later Grijns [1927] was able to obtain very minute plaques on meat extract sucrose agar with the lytic principle of clover nodule organisms, thus establishing the true bacteriophagic nature of the lytic principle. Hitchner [1930] was unable to verify the work of Grijns with the lytic principle isolated by him and all his attempts to produce lysis on solid media were unsuccessful. Thus in the attempts of previous workers to find the nature of the lytic principle the formation of plaques was an exception rather than a rule so far as the lytic principles of the root nodule organisms of leguminous plants were concerned. Working with the lytic principles isolated from the roots of *Trifolium alexandrianum*, *Lathyrus odoratus*, *Dolichos Lablab* and others at Pusa, the formation of plaques has been invariably observed except with bacteriophages of very weak virulence. The production of lysis on solid media was found to be quite regular. The medium usually employed was medium E with 2 per cent. agar. To demonstrate the plaques, the active filtrate was diluted according to its virulence and mixed with a heavy suspension of the corresponding organisms. This was spread on a poured agar petri dish. The plate was then incubated at 30°C. The formation of plaques was quite distinctly visible after twenty-four hours. Two types of plaques were obtained in the course of our investigation. One type of plaque (Plate XIII, fig. 1) was usually quite small at the time of formation, showing a circular patch of $\frac{1}{2}$ to 1 mm. in diameter quite bare of any growth. This small patch later got appreciably large (4-5 mm. diameter) and was soon surrounded by a larger circular zone of bluish transparent growth which was quite flat on the surface of the agar as opposed to raised, pearly, white, slimy growth in other parts of the plate. On further incubation this zone of bluish transformation slowly increased and when a large number of plaques were present the whole plate soon assumed a transformed appearance (Plate XIII, fig. 2 and Plate XIV). If a drop of the undilutated active filtrate was mixed with the suspension and spread on a plate there was no regular plaque formation but the whole plate assumed the transformed bluish appearance with large patches of agar devoid of any growth, after 24 hours. When the suspensions of the organisms were inoculated with a platinum needle which had been brought in contact with the central bare space of the plaque, and incubated, the dissolution of the cultures was observed after twenty-four hours, thus showing that the clear central spaces

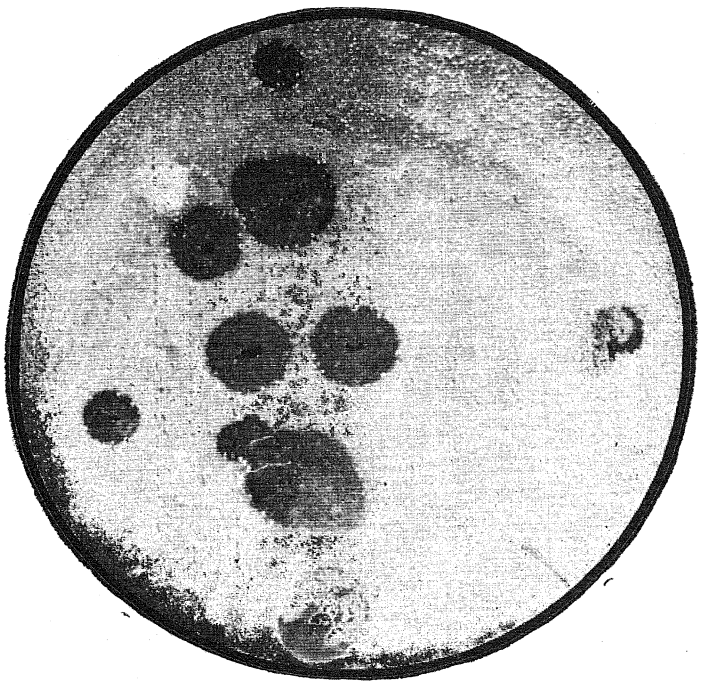


Fig. 1. Bacteriophage from *Trifolium alexandrianum* on organisms from the Trifolium plants 1×10^{-6} dilution. After 4 days.

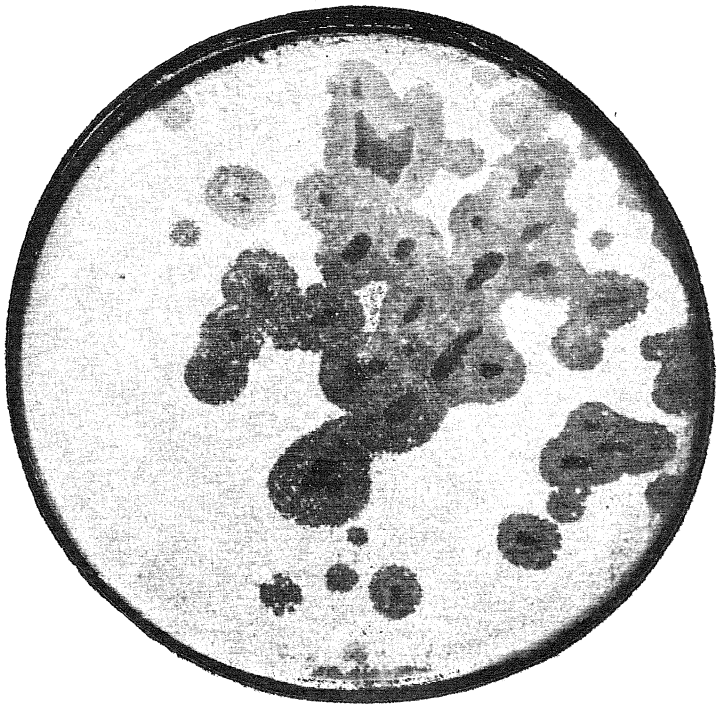
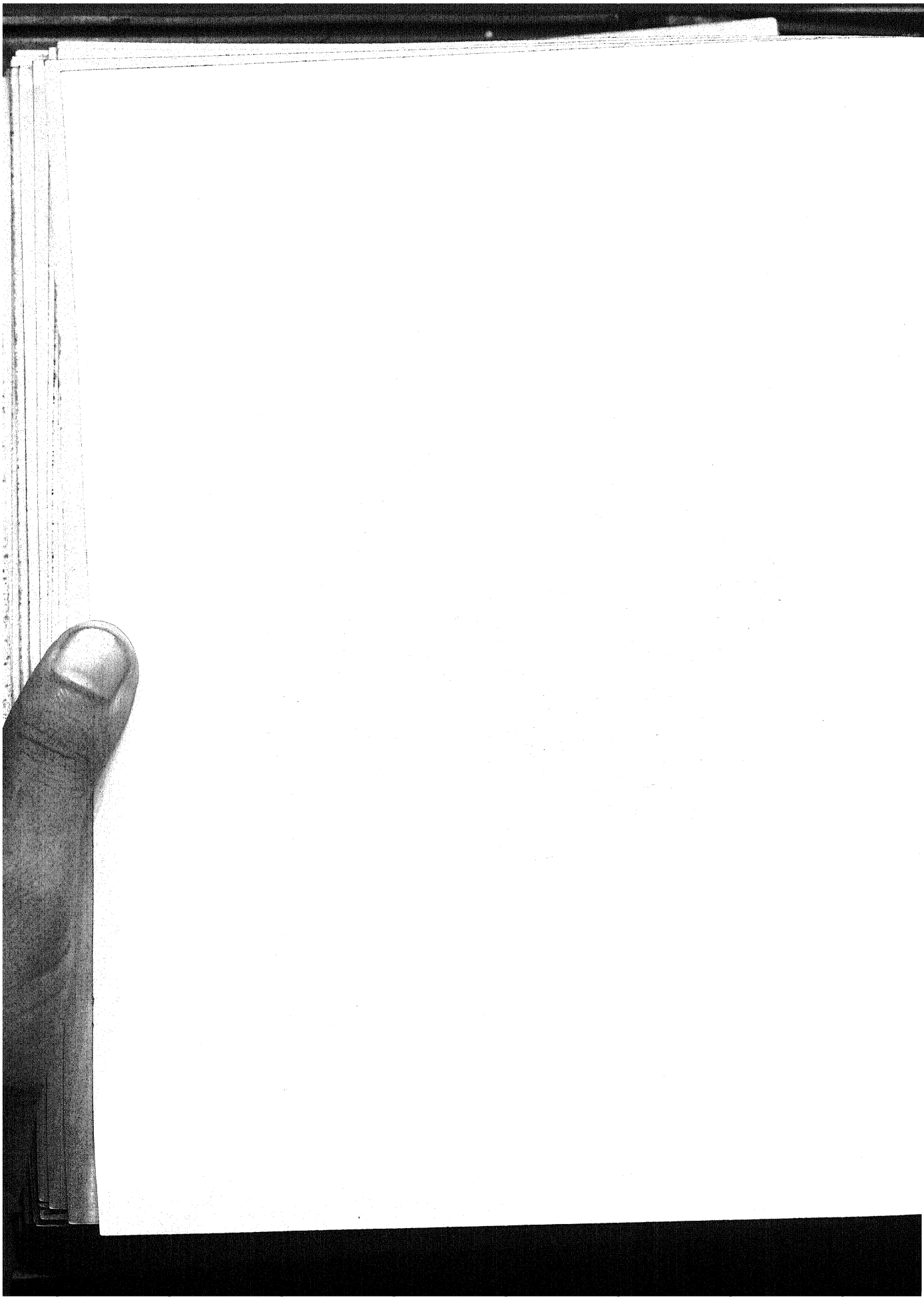


Fig. 2. Bacteriophage isolated from *Trifolium alexandrianum* on organisms from Trifolium plants 1×10^{-6} dilution. After 6 days.





Bacteriophage from *Trifolium alexandrianum* on organisms from Trifolium plants 1×10^{-6} dilution. After 10 days.

PLATE XV.

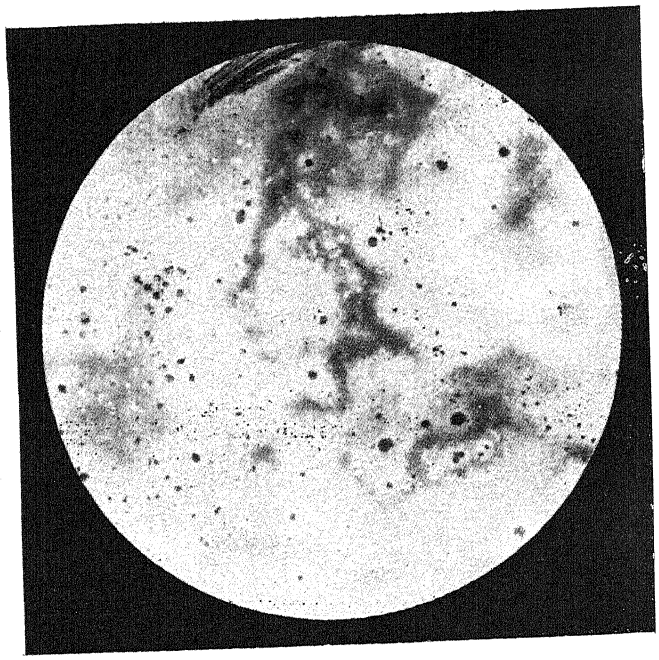


Fig. 1. Bacteriophage isolated from *Dolichos Lablab* on organisms from Dolichos 1×10^{-6} dilution after 4 days.

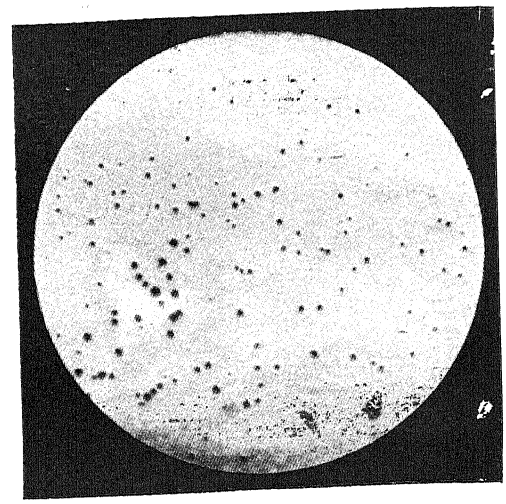


Fig. 2. Bacteriophage from *Dolichos Lablab* trained for organisms from Trifolium plants by two passages at the expense of organism from Trifolium plants. On organisms from Trifolium plants 1×10^{-6} dilution after 4 days.

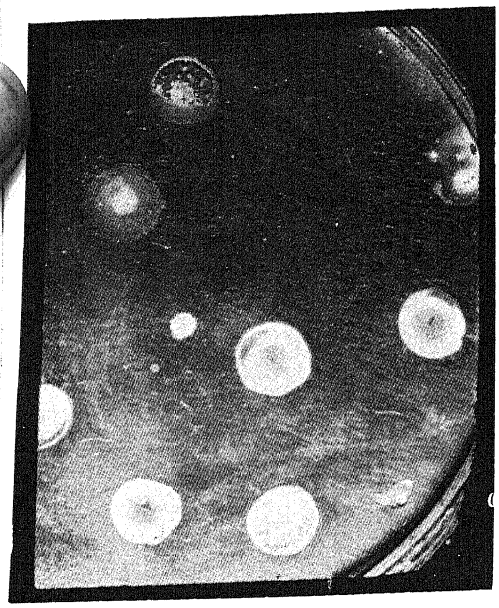


Fig. 3. Bacteriophage from *Trifolium alexandrianum* on colonies of organisms from Trifolium plants. 1. Top colony half transformed. 2. Below that whole colony transformed. 3. Two colonies in which the transformation has just started. 4. Bottom two colonies in which one is not yet attacked and one showing the transformation starting from the centre.

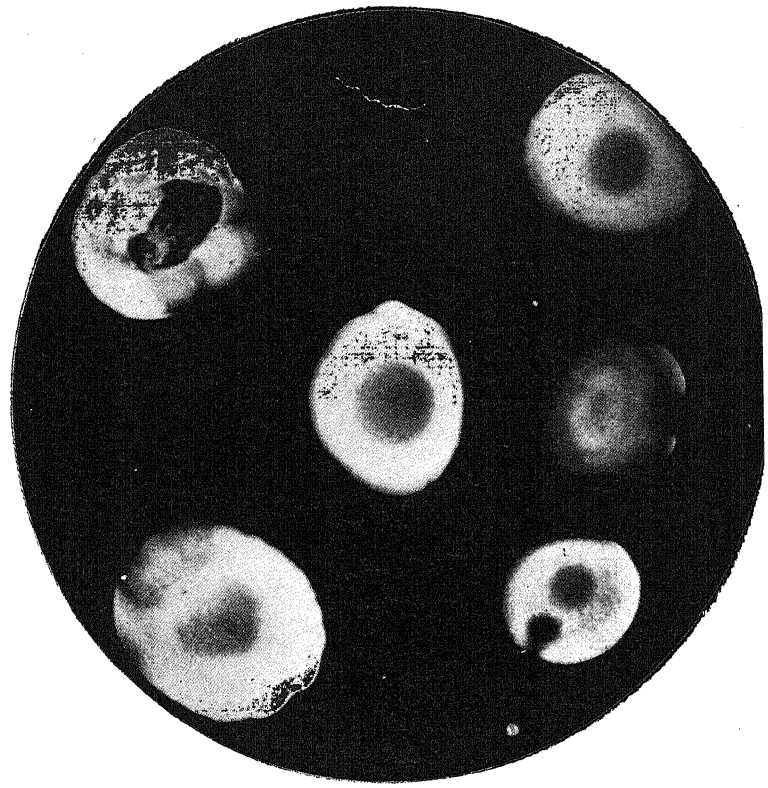


Fig. 4. Lathyrus organism by its bacteriophage. 1. Top two colonies showing transformation—one with part of it transformed, other showing the beginning of the attack on the margin. 2. Middle two colonies. Left one showing the beginning of the transformation and the right one showing almost complete transformation. 3. Bottom two colonies showing part transformation.

were the true colonies of the bacteriophage. When the suspensions were inoculated with the growth from the bluish transparent zone no clearing of the suspensions was observed but the growth in the inoculated tubes developed a bluish transparent appearance with a slight flocculation of the growth after a few days. When these bluish cultures were filtered through porcelain filter candles the filtrates showed the presence of the bacteriophage as was seen by the dissolution of liquid cultures as well as by the plaque formation on solid media. When similar experiments were conducted with the normal growth of the culture from the same plate no lytic principle could be found. It was definitely established that the bacteriophages giving this type of plaques had an unique property of slowly spreading into the culture from the first formed plaques and dissolving the organisms and the slime in the surrounding area. The dissolution of the organisms is not, however complete, as the bluish growth persists. This lytic action was accompanied by a change in the appearance of the culture from raised, opaque, pearly white, slimy and glistening growth to flat, transparent bluish and slightly granular growth without any slime, which contained organisms resistant to lysis by the bacteriophage.

The second type of plaques (Plate XV, figs. 1 and 2), which were usually met with in strains which produced little or no slime, were quite ordinary circular patches quite devoid of any growth. The rapid spreading which characterised the first type of plaques was not observed in the second type though some slight spreading could be seen after many days (more than a week) by means of a magnifying glass. As in the case of the first type of plaques, the bare space in these plaques proved to be the true colonies of the bacteriophage. Inoculation from these spaces into fresh cultures demonstrated lysis in liquid cultures and plaque formation on solid media. The growth near the plaques showed absence of the bacteriophage as neither lysis nor plaque formation was observed when it was added to a fresh culture of the organisms. It may be observed that the two types of plaques described above occurred respectively in slimy and non-slimy strains of the nodule organisms. To investigate whether the plaques were innately different or varied with the nature of the growth of the organisms, bacteriophage giving typical plaques of the second type, occurring with the non-slimy strain, was successively passed through a strain which was slimy, and it was observed that after a few passages it gave typical plaques of the first type. The experiments were repeated with special care to avoid chance contamination of the bacteriophage giving the plaques of the first type. At each passage the filtrate was diluted and plated, and the inoculations for the second passage were made from isolated typical plaques of the second type. The typical plaques of the second type decreased with successive platings and many plaques began to show a slight zone of secondary transformation round them. The

zone of this transformation increased gradually with successive passages and ultimately the plaques could hardly be differentiated from the typical plaques of the first type.

These experiments definitely showed that the bacteriophages giving two different types of plaques were not in fact inherently different but that some bacteriophages had developed the power of spreading and dissolving the slime while others had that power latent and developed it only under suitable conditions.

Gratia [1931, 2] in a very recent paper has shown the occurrence of the two kinds of bacteriophages for *Staphylococcus* giving two types of plaques very similar to the two types mentioned in this paper. But in view of the observation made in our experiment described above that the bacteriophage giving one type of plaques may be made to give plaques exactly like the other type when grown with a suitable strain, it is doubtful whether there are in reality two different bacteriophages for the nodule organisms and a suggestion may be ventured that the two types of plaques observed by Gratia may be really due to different kinds of growth of the organisms with which the bacteriophages were previously cultured.

(*b*) *The Twort phenomenon.*—Another phenomenon which was met with while working with these bacteriophages was the appearance of the transformation of the growth of the organisms in a colony on an agar plate. For this purpose an old colony of the organism on a plate was touched with the bacteriophage. The colony after some time began to turn flat, bluish and transparent from one or several points on the periphery. This was accompanied by the transformation from a raised, slimy appearance to a flat slightly granular one, quite distinct from the normal appearance. This transformation continued further towards the centre of the colony and ultimately the colony turned completely flat, bluish, granular and semi-transparent. Sometimes this effect took place in concentric rings from the periphery. However, when the filtrate was highly virulent the transformation began from the point of touch. The transformation was, however, not always regular as it spread towards the centre. Ultimately, however, the whole colony was transformed (Plate XV, figs. 3 and 4). Other normal colonies in the same plate though not touched by the bacteriophage soon got the infection of the bacteriophage as seen by the similar transformation starting in their appearance.

In order to see whether the changed appearance of colonies was due to the dissolution of the organisms, the numbers of organisms in the bacteriophaged colonies were counted and compared with those in colonies kept as control in another petri dish. For this purpose a single colony was planted in a number of petri dishes and after they had developed for two days, some were touched with a minute quantity of the bacteriophage. When the appearance of the

colony touched with the bacteriophage was completely transformed, the agar round about the colony was cut with a sterile knife and the colony, with the agar, transferred into sterile water, and an emulsion prepared by violently shaking for 10 minutes so as to disintegrate the agar completely. Dilutions were made and plated and the number of organisms calculated for the whole of the colony. For comparison counts were made from the colonies kept as controls, after similar treatment. The average number of organisms in the control colonies was found to be 3,200,000,000, while in the phaged colonies the number was found to be 300,000. The appearance of all the colonies on the agar plates poured as described above from phaged colonies was quite normal for two days, after which they began to show transformation similar to that of the phaged colony. The transformation appeared in regular concentric rings. This bluish transformation of colonies in the plates poured from the colony was proved to be due to the bacteriophage by filtering the emulsion of the colonies through a porcelain filter candle. The filtrate was found to bring about the lysis of the organisms in liquid cultures as well as the transformation of the colonies of the organisms.

This unique property of transformation of the colony with partial dissolution of the organisms may be attributed to the spreading nature of these bacteriophages which, probably with the associated enzyme, decomposed the slime also. This transformation has a resembalance to the phenomenon first observed by Twort [1915]. In Twort's phenomenon the bacteriophage dissolved away the organisms leaving the bacterial fragments staining red with giemsa in the vitreous mass. In the transformation phenomenon described above slime and bacteria were dissolved away leaving a very few bacterial fragments, with the resistant organisms, and some old dead organisms on the agar.

D' Herelle had not been able to initiate a visible dissolution of the organisms in a colony with the bacteriophages he had isolated and hence he distinguished his phenomenon of bacteriophagy from that of Twort. Recently D'Herelle [1931] has come to recognize the spreading nature of some of the bacteriophages on solid media and the only distinction he makes is the formation of fine granules staining reddish with giemsa in the vitreous transformation. D'Herelle in his attempts to differentiate the two phenomena has laid unnecessary stress on this formation of bacterial fragments staining reddish with giemsa stain. He recognizes that, if the process of bacteriophagy in liquid culture is observed under the microscope, the bacterial cells first get poorly stained until finally very rarely a cell or two may be found stained. He further observes, " at the same time amorphous debris and granulations, derived most certainly from the bacteria already dissolved, are seen. These granulations dissolve more slowly than the remaining portions of the bacterial protoplasm; gradually the formless debris disappears and in turn the granules ". If

the same process of bacteriophagy were to take place on solid media it is natural to suppose that these debris and granulations, which disappear, due to the rapid action of enzymes from ruptured bacterial cells, after thirty-six hours in liquid culture, take a longer time to disappear on solid media; hence the debris and granulations are seen on solid media especially when the action of bacteriophages is not inhibited by the products of bacterial dissolution.

Gratia [1931, 1] working with the gram positive *Staphylococcus* bacteriophages has demonstrated that the resistant organisms were gram positive and the vitreous mass gram negative, and that the vitreous material showed fine irregular particles on staining according to Borrell's method. As the nodule organisms are gram negative Gratia's gram staining method was found to be unsuitable for differentiating the resistant organisms from the vitreous material. The staining of the transformed culture according to the method of Borrel showed that there were some fine particles mixed with a few normal sized bacteria. Staining of the transformed culture in the earliest stages by giemsa stain showed very small irregular particles staining red, mixed with very few organisms. Also in a smear made from the centre of a plaque, where there was no visible growth, similar particles staining red with giemsa were seen. When a smear was made from a transformed colony which had remained so for some time, granules staining red with giemsa were few and hard to distinguish from granules occurring in the bacterial cells, as the cell wall did not stain prominently. The staining reactions of organisms from the resistant secondary cultures and the transformed cultures were similar to those of the organisms of the original culture without the action of the bacteriophage, and could not be distinguished from each other by any such method of differential staining.

Secondary growth.

Secondary growth occurred in all the liquid cultures which had become clear by the action of the bacteriophage. The phaged cultures remained clear normally for two days but some phaged cultures became slightly turbid only after a week while others turned turbid almost immediately after they had cleared. The secondary growth was observed to grow much more quickly in cultures phaged by a bacteriophage of weak virulence, than in cultures phaged by one of strong virulence. The secondary growth generally remained at the bottom of the tube or adhered to the test tube in agglutinated clusters, and the liquid medium in the tube assumed a bluish tint. When the secondary growth was plated it generally gave normal looking colonies of the organisms, but after three days or more, bluish rings were observed on the outside border of the colonies. These rings broadened till all the colonies assumed the transparent bluish appearance of a phaged colony. When one

of the bluish transparent colonies was transferred to the liquid medium, and filtered through a candle after a growth of three to five days in that medium the filtrate was found to contain the lytic principle which readily acted on the susceptible strains. The growth of the secondary cultures on solid media was markedly different from the growth of the ordinary strains. They grew poorly and had not the white, glistening, raised, and slimy appearance of normal growths.

The altered growth was highly characteristic of the action of the bacteriophage and could be easily distinguished, being slight, flat, slightly granulated with bluish patches and without any slime. When the secondary culture was repeatedly plated on a medium rich in sugar like Ashby's mannite agar, the number of colonies having the bluish rings associated with the presence of the bacteriophage diminished with successive platings. Quite normal looking colonies which had shown no bluish ring formation for fourteen days often gave rise to colonies with bluish rings on a second plating. From this appearance of bluish rings on the second plating it is concluded that the bacteriophage may remain associated with the organisms without any apparent manifestation of its action on media rich in sugar. When such a mixed culture of the organisms already containing a bacteriophage, though not showing any apparent sign, as noticed above in some colonies of the first plating on Ashby's mannite, is exposed to the action of another bacteriophage, it is found that the added phage is unable to show visible action on the mixed culture. If the mixed culture which was normal on Ashby's mannite was transferred to the usual marmite mannite medium containing 0·2 per cent. sugar the presence of the lytic principle was soon made manifest by the formation of the characteristic flat slimeless growth. It was found difficult to obtain ultra-pure cultures of the organism from the secondary growth. Usually the method followed was successive plating on Ashby's mannite. Three platings were sufficient to eliminate the bacteriophage if transfers of selected colonies were made. Most of the colonies at this stage gave normal cultures on the marmite mannite agar and the contamination of the bacteriophage seemed to have been eliminated. The cultures of the strain thus freed from the contamination behaved like the original susceptible strain as regards lysis. If the secondary culture was transferred to Ashby's mannite in slants for few transfers the contaminating phage instead of being easily separated was difficult to eliminate from the culture. The growth of such cultures on marmite mannite appeared to be normal for some transfers and then it suddenly changed to the characteristic growth of the contaminated culture. The secondary cultures which had remained longer on Ashby's mannite retained the normal appearance longer on marmite mannite agar. The presence of the lytic principle in such normal looking culture was easily manifested by transferring it into a suspension of the susceptible organisms in liquid medium containing 0·2 per cent. sugar or without any sugar and filtering through a candle

after 2 days. The filtrate acted on the original susceptible strain but failed to produce visible lysis in the suspensions of the contaminated culture from which it was derived. When the contaminated culture was subjected to the action of the bacteriophage of higher virulence than that associated with the culture, the lytic action was seen, though not to the degree reached in uncontaminated culture.

Specificity of the bacteriophages.

When the lytic principles were weak in virulence many of the strains of the organisms appeared to remain unaffected by them; with the increase of virulence at the expense of one susceptible strain by successive inoculation with the phage, all the other strains of the organisms isolated from the corresponding species of plants that were tested were found to be attacked and dissolved by the virulent lytic principle. Though all strains of the organisms were affected by the bacteriophage the degree of lysis varied from strain to strain. Some strains were dissolved within 24 hours while others flocculated and turned bluish in the liquid media after a week. On agar, the colonies of all these strains, however, showed the bluish characteristic transformation of their appearance if touched with phage. In some cases there was no visible clearing nor any flocculation or bluish transformation in liquid cultures, the colonies of these strains if touched with the virulent bacteriophage invariably turned bluish and transparent with the characteristic flattening of the raised growth. The time taken for the transformation of these cultures to begin was much greater than that for the transformation of normal strains. If the phaged colony of the strain which showed no visible lysis in liquid medium was transferred to a liquid medium and filtered through a candle, the filtrate, after a couple of such transfers, at the expense of that apparently resistant strain on solid medium began to show visible lysis in the liquid medium. Thus the bacteriophage was able to adapt itself to attack the strain by training to live on that strain. The size of the plaques varied in different strains of the corresponding organisms. It was found that the plaques decreased in size if the bacteriophages were plated with organisms which more or less showed a resistance to lysis. Sometime they assumed almost microscopic size and could not be well differentiated.

TABLE III.

Specificity of the bacteriophages for the nodule organisms derived from different species of leguminous plants.

Plants from which the organisms were derived	Bacteriophage isolated from *Trifolium alex.* 1×10^{-8}				Bacteriophage isolated from *Lathyrus odoratus* 1×10^{-8}				Bacteriophage isolated from *Dolichos Lablab* 1×10^{-10}			
	Liquid culture	Plaques	Dissolution in colony	No. of passages after which dissolution was seen in liquid cultures	Liquid culture	Plaques	Dissolution in colony	No. of passages after which dissolution was seen in liquid cultures	Liquid culture	Plaques	Dissolution in colony	No. of passages after which dissolution was seen in liquid cultures
	At start				At start				At start			
Trifolium alex.	++++	Large	After 2 days	None	+	Very minute	After 5 days	2	+	Very minute	After 5 days	3
Lathyrus odoratus	++	Small	After 5 days	2	+++	Very large	After 2 days	None	+	—	After 7 days	4
Dolichos Lablab	—	—	—	7	—	—	—	7	++++	Large	5	None
Pisum sativum	+	—	After 10 days	4	+	—	After 10 days	4	—	—	—	7
Crotolaria juncea	—	—	—	8	—	—		8	—	—	—	8
Cicer arietum	—	—	—	7	—	—	—	7	—	—	—	7
Cajanus indicus	+	—	After 10 days	5	+	—	After 10 days	5	—	—	—	8
Soja, max.	—	—	—	12	—	—	—	11	—	—	—	12
Madicago sativa	—	—	After 15 days	5	—	—	—	7	—	—	—	8
Sesbania aculeata	—	—	—	8	—	—	—	8	—	—	—	9
Vigna catjang	—	—	—	7	—	—	—	7	—	—	—	9
Phaseolus actinifolius	—	—	—	6	—	—	—	8	+	—	After 5 days	6

Thus our experiments show that the specificity of the bacteriophages is not limited to the particular strain of organisms. The partial resistance of some of the strains may well be explained by their long association with some lytic principle which had resulted in their acquiring a resistance by constant contact with it.

Passing from the specificity of the bacteriophages for the strains of the organisms derived from the same species of plant, further experiments were conducted to determine whether the bacteriophages dissolved the organisms isolated from leguminous plants of species other than the one from the nodules of which the bacteriophages were isolated. For the cross inoculation experiments we had three tests to find out whether any particular bacteriophage acted on the organisms or not.

1. Inoculation of the phage and the organisms to be tested in a liquid medium for observing the clearing of the medium.

2. Simultaneous inoculation of the phage and the organism on a solid medium to see plaque formation.

3. Inoculation of the phage on to colonies of the organisms, already grown on agar.

Sometimes the bacteriophages had to be trained to act on the organisms by inoculating the phage in the culture of the organisms to be tested, filtering the tubes receiving bacteriophage, and reinoculating the filtrate in the suspension of the same organisms. The results, which are set forth in Table III showed that the bacteriophages tested were polyvalent and could attack or be trained to attack practically all the organisms occurring in the nodules of the different species of the leguminous plants.

Inactivation of the bacteriophages.

The bacteriophages were found to be heat labile, a temperature of 70° to 75°C, for five minutes served to inactivate them. This was clearly demonstrated by the following experiments. Six tubes containing 10 c.c. sterile water were taken; two drops of active bacteriophage added to each of the tubes. These tubes were placed in a water bath which was heated to a particular temperature and kept at that temperature for the requisite length of time. After cooling, one c.c. of the dilute heated principle was added to freshly prepared suspensions of the organisms and incubated,

The experiments were conducted in triplicates. The results are set forth in Tables IV and V.

TABLE IV.

Effect of temperature on inactivation of the Trifolium alex. *bacteriophage and* Lathyrus ordoratus *bacteriophage.*

Temperature	Length of time	Amount of active filtrate	After 24 hours	After 48 hours	After 72 hours
30°C	0 min.	1/100	Clear	Clear	Clear
50°C	15 min.	,,	,,	,,	,,
60°C	,,	,,	,,	,,	,,
65°C	,,	,,	Slightly clear	,,	,,
70°C	5 min.	,,	,,	Slightly clear	,,
75°C	,,	,,	Cloudy	Cloudy	Cloudy
80°C	,,	,,	,,	,,	,,

TABLE V.

Effect of temperature on inactivation of the Dolichos Lablab *bacteriophage.*

Temperature	Length of time	Amount of active filtrate	After		
			24 hours	48 hours	72 hours
Control . . .	0 min.	1/100 c.c.	Clear	Clear	Clear
50°C	15 min.	,,	,,	,,	,,
60°C	,,	,,	,,	,,	,,
65°C	,,	,,	Slightly clear	,,	,,
70°C	5 min.	,,	Cloudy	Cloudy	Cloudy
75°C	,,	,,	,,	,,	,,
80°C	,,	,,	,,	,,	,,

THE PROGRESSIVE RATE OF THE LYTIC ACTION.

In order to determine the rate of lytic action and the diminution of the organisms in the cultures, a series of experiments were conducted in which the following procedure was adopted.

Flasks containing 100 c.c. of marmite mannite broth containing 0·2 per cent. sugar were inoculated with the organisms by adding an equal inoculum in each flask. These were incubated for 24 hours when turbidity was appreciably developed. To one batch of flasks 0·2 c.c. of the active bacteriophage was added, while to the other batch the same amount of the active filtrate, inactivated by heating to 100° C for 5 minutes on a water bath, was added. Numerical counts of the organisms in these flasks were then made by plating out on marmite mannite agar immediately after inoculation and at different intervals during the period of lysis.

The results of four typical experiments with *Trifolium* organism and its phage are given in Table VI.

TABLE VI

Rate of dissolution of the organisms from Trifolium *plants by the bacteriophage from* Trifolium alexandrianum.

(Number of organisms per c.c. in thousands)

Time	Bacteriophage No. 4 virulence 1×10^{5}		Bacteriophage No. 9 virulence 1×10^{-6}		Bacteriophage No. 14 virulence 1×10^{7}		Bacteriophage No. 19 virulence 1×10^{8}	
	Control	Receiving bacteriophage	Control	Receiving bacteriophage	Control	Receiving bacteriophage	Control	Receiving bacteriophage
Start	41,000	38,000	25,400	22,700	29,300	34,000	54,000	46,000
3 hours	86,000	61,000	78,900	55,500	84,000	93,000	93,000	1,04,000
7 „	93,000	8,800	88,000	6,070	1,02,000	5,000	1,32,000	2,000
11 „	1,41,000	5	1,29,000	3	1,34,000	2	1,84,000	$\frac{1}{2}$
24 „	2,01,000	39	1,98,000	1	2,04,000	$\frac{1}{2}$	2,24,000	$\frac{1}{10}$
30 „	2,40,000	101	2,36,000	6	2,28,000	$\frac{1}{2}$	2,65,000	$\frac{1}{10}$
48 „	3,84,000	113	3,76,000	18	4,26,000	$\frac{1}{2}$	4,40,000	$\frac{1}{8}$
72 „	5,02,000	250	4,88,000	118	4,89,000	4	4,92,000	1

In the experiments cited it may be noticed that the organisms were at no time completely destroyed even when the bacteriophage was very virulent and no turbidity was visible in the flasks containing the bacteriophage. Plates made during the period of active lysis contained some colonies which were abnormal. Colonies exhibiting the abnormal appearance described previously looked like normal colonies for a period of two or three days after which they developed the typical blue transparent transformation from the periphery which soon extended to the centre

in concentric rings. The number of such colonies increased with time and after the end of the active lysis all the colonies developed these transformations. The progressive rate of lysis observed with *Lathyrus* organisms and their bacteriophage as well as *Dolichos* organisms and their bacteriophage gave similar results to those described for the *Trifolium* organisms.

Summary.

A method has been evolved by which bacteriophages have been isolated from roots of many leguminous plants. Various media have been tried and it has been established that media suitable for isolation of the bacteriophage are unsuited for enhancement of virulence and *vice versa*. The formation of plaques has been invariably observed. Two types of plaques have been met with. The formation of two different types of plaques depended upon the kind of the growth of the organisms and the previous history of the bacteriophages and not on the inherent nature of bacteriophages. The bacteriophage giving one type of plaques may be made to give plaques exactly like the other type when grown with a suitable strain of the organisms showing that all bacteriophages of the nodule organisms represent one primary bacteriophage.

Bacteriophage of these organisms showed a modified form of Twort's phenomena which took place when a colony of the organisms was touched with the bacteriophage. The appearance of the colony changed from raised, opaque, pearly white, slimy and glistening one to flat, transparent, bluish and slightly granular one. The bacteriophages were not particularly specific in their action. Not only all the strains of the corresponding organisms were attacked but the bacteriophages could be trained to attack any organism derived from widely different species of leguminous plants. The bacteriophages were heat labile and a temperature of 75°C. for 5 minutes served to inactivate them. The progressive rate of lytic action showed that organisms were at no time completely destroyed even when the bacteriophage was of maximum virulence. Secondary growth occurred in all liquid cultures which had become clear by the action of the bacteriophage. Secondary cultures showed some resistance to the action of the bacteriophage but they lost this resistance if cultivated for one generation in the absence of the phage. The separation of the organisms from the bacteriophage was difficult; only repeated plating was successful if the contact of the organisms with the bacteriophage was not of a long duration.

My thanks are due to Mr. J. H. Walton, Imperial Agricultural Bacteriologist, at whose suggestion this investigation was undertaken and to Mr. N. V. Joshi for his assistance in the preparation of this paper.

REFERENCES.

D'Herelle, F. (1926) . . . . . The Bacteriophage and its Behavior.
——————(1931) . . . . . *Ann. De L'inst. Past.* **46**, 616.
Gerrelsen, F. C., Grijns, A., Sack, T., Sohngenm, N. L. (1923) *Centbl. Bakt.* 2 Abt., 60 B, 311.
Gratia, A. (1931, 1) . . . . . *Ann de L'inst. Past.* **46**, 619.
——————(1931, 2) . . . . . ,, ,, ,, 622.
Grijns, A. (1927) . . . . . *Centbl. Bakt.* 2 Abt., 71 B, 248.
Hitchner, E. R. (1930) . . . . . *J. Bact.* **19**, 191.
Joshi, N. V. (1920) . . . . . *Mem. Dept. of Agric. India, Bact. Ser.*, **1**, 247.
Twort, F. W. (1915) . . . . . *Lancet*, **2**, 1241.

A STATISTICAL NOTE ON CERTAIN RICE-BREEDING EXPERIMENTS IN THE CENTRAL PROVINCES.

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1. In the June (1931) issue of this *Journal*, Messrs. D. N. Mahta and B. B. Dave gave a most interesting account of "Rice-breeding in the Central Provinces"*. The experimental work done by the authors and the results achieved by them are likely to be of far-reaching importance to Indian Agriculture. I may, therefore, be excused for offering a few remarks on the statistical methods used by the authors to test the significance of their results.

The authors raised a number of new varieties by hybridization. These hybrids (which possessed the desirable characters) were "tested for yield among themselves and against their parents. The testing of yield was carried out by the Latin Square method." The yield of 13 varieties replicated 13 times in plots ($4' \times 4'$) were recorded (page 361). The Table I giving the primary data (yield in ounces of each strain noted below the varietal number) is reproduced here (from page 361) for convenience of reference.

* *Ind. J. Agric. Sci.*, Vol. I, Part III, June 1931, pp. 351—371. Subsequent page references refer to this paper.

TABLE I.

(*Yield. Data from the* Indian Journal of Agricultural Science, *Vol. I, Part III, Page 361.*)

B. P. 22/1	Parewa	B. S. 23	B. P. 15	B. S. 30	Bhondu	B. S. 24	Surmatia	B. P. 19	B. P. 22/2	B. P. 12	B. P. 20	B. P. 11
19·5	20·0	28·0	33·75	25·5	31·0	29·5	22·75	33·0	27·75	26·5	28·75	29·5
Bhondu	20	19	23	11	22/2	Parewa	24	30	22/1	15	Surmatia	12
19·5	16·0	21·0	18·25	19·0	18·25	21·5	25·0	13·25	19 0	22·5	18·25	24·5
12	22/2	Surmatia	30	Parewa	24	22/1	19	20	11	23	Bhondu	15
25·5	21·0	22·0	25·5	23·0	27·75	30·25	25·5	27·5	21·75	27·0	29·25	32·0
24	30	12	11	23	15	Surmatia	22/1	Bhondu	19	Parewa	22/2	20
23·0	21·75	26·0	27·0	25·75	25·5	21·75	27·0	27·75	25·25	24·0	24·5	27·25
19	Surmatia	24	Bhondu	22/1	30	15	22/2	Parewa	12	20	11	23
26·5	17·25	28·5	28·0	27·5	25·5	25·75	23·25	26·0	25·0	28·5	28·25	28·25
22/2	24	Bhondu	Parewa	Surmatia	22/1	23	12	11	20	19	15	30
25·75	24·0	25·5	21·0	20·3	27·25	26·25	24·25	19·0	25·0	26·5	25·25	21·0
Parewa	23	22/1	Surmatia	20	12	30	Bhondu	22/2	15	11	19	24
22·75	25·5	27·0	20·0	23·5	23·0	20·5	21·25	23·25	27·0	24·0	26·0	29·25
20	11	30	24	22/2	Parewa	19	15	12	23	Surmatia	22,1	Bhondu
26·5	18·0	15·5	25·5	19·5	21·0	23·0	19·25	23·5	23·75	21·0	27·5	29·75
15	Bhondu	11	22/2	12	19	20	23	22,1	Parewa	30	24	Surmatia
21·5	22·75	19·0	20·75	22·75	17·5	20·5	26·0	24·75	20·5	21·25	30·0	21·5
23	22/1	15	20	19	11	12	Parewa	Surmatia	24	Bhondu	30	22/2
25·5	29·0	16·5	23·0	18·25	24·0	26·0	22·0	22·5	31·25	29·0	25·5	21·5
11	19	Parewa	12	24	20	Bhondu	30	15	Surmatia	22/2	23	22/1
23·25	20·75	15·0	25·5	25·0	24·75	27·25	21·75	29·0	27·5	26·0	31·5	31·5
30	12	20	22/1	15	Surmatia	22/2	11	23	Bhondu	24	Parewa	19
20·75	20·75	21·0	25·75	25·75	21·25	25·75	26·5	29·5	28·5	29·0	24·5	22·25
Surmatia	15	22/2	19	Bhondu	23	11	20	24	30	22/1	12	Parewa
16·75	24·75	21·5	13·0	22·0	30·5	22·5	21·5	26·75	20·0	22·25	24·5	19·25

In discussing the significance of the results the authors calculated the mean and standard deviations (based on samples of 13), and used the classical theory of errors to test the differences in yield.

2. A more recent statistical procedure can, however, be adopted with advantage and will lead to a greater precision in the quantitative interpretation of the results. This is attained in two ways. First by the use of the appropriate theory of small samples.* Secondly, by eliminating the effect due to soil heterogeneity by using Fisher's method of "analysis of variance", a procedure which, very fortunately, can be adopted in the present case owing to the use of the Latin Square arrangement. It is desirable that agricultural experimenters in India should make themselves familiar with Fisher's method. Full details of the numerical calculations are, therefore given below.†

* The application of the classical theory of errors (which was developed on the assumption of large samples) will not yield absolutely correct results in the case of small samples. This difficulty can be met by using Fisher's t-test. I have discussed this question in a recent note of the *Indian Journal of Agricultural Science* (February, 1932) which may be referred to for full details.

† I am intentionally confining my remarks to the explanation of the actual procedure of numerical calculations. Excellent descriptions of the method will be found in R. A. Fisher's 'Statistical Methods for Research Workers' (3rd edition, 1930, Chap. VIII). J. O. Irwin has recently given a resume of the underlying theory in an article on "Mathematical Theorems Involved in the Analysis of Variance" in the *Journal of the Royal Statistical Society*, Vol. XCIV, 1931, Part II, p. 284.

There are three Arithmetical slips in the numerical calculations in Mahta and Dave's paper. There is a serious mistake in 'B.×S. No. 24', Serial No. 13 (p. 367). The yield is printed as 19·50; the correct figure (from Table I) is 29·50. The corresponding square should be changed from 380·2 to 870·25.

For 'Bhondu' (p. 368) serial number 4, the yield is printed as 27·75. The correct value is 27·25. The total yield of Bhondu has, however, been evidently calculated with the figure 27·25). The square 742·7 should be corrected to 742·56.

For 'Surmatia', serial No. 8, the printed figure is 20·50; the correct figure should be 20·33. The square should also be changed from 420·4 to 413·31.

TABLE II.

Yield in ounces minus *24 oz.*

(Derived from Table I.)

		1	2	3	4	5	6	7	8	9	10	11	12	13	Total for rows.
	1	—4·50	—4·00	+4·00	+6·75	+1·50	+7·00	+5·50	—1.25	+9·00	+3·75	+2·50	+4·75	+5·50	+40·50
	2	—4·50	—8·00	—3·00	—5·75	—5·00	—5·75	—2·50	+1·00	—10·75	—5·00	—1·50	—5·75	+0·50	—56·00
	3	+1·50	—3·00	—2·00	+1·50	—1·00	+3·75	+6·25	+1·50	+3·50	—2·25	+3·00	+5·25	+8·00	+26·00
	4	—1·00	—2·25	+2·00	+3·00	+1·75	+1·50	—2·25	+3·00	+3·75	+1·25	..	+0·50	+3·25	+14·50
	5	+2·50	—6·75	+4·50	+4·00	+3·50	+1·50	+1·75	—0·75	+2·00	+1·00	+4·50	+4·25	+4·25	+26·25
	6	+1·75	0·0	+1·50	—3·00	—3.67	+3·25	+2·25	+0·25	—5·00	+1·00	+2·50	+1·25	—3·00	—0·92
	7	—1·25	+1·50	+3·00	—4·00	—0·50	—1·00	—3·50	—2·75	—0·75	+3·00	..	+2·00	+5·25	+1·00
	8	+2·50	—6·00	—8·50	+1·50	—4·50	—3·00	—1·00	—4·75	—0·50	—0·25	—3·00	+3·50	+5·75	—18·25
	9	—2·50	—1·25	—5·00	—3·25	—1·25	—6·50	—3·50	+2·00	+0·75	—3·50	—2·75	+6·00	—2·50	—23·25
	10	+1·50	+5·00	—7·50	—1·00	—5·75	..	+2·00	—2·00	—1·50	+7·25	+5·00	+1·50	—2·50	+2·00
	11	—0·75	—3·25	—9·00	+1·50	+1·00	+0·75	+3·25	—2·25	+5·00	+3·50	+2·00	+7·50	+7·50	+16·75
	12	—3·25	— 3·25	—3·00	+1·75	+1·75	—2·75	+1·75	+2·50	+5·50	+4·50	+5·00	+0·50	—1·75	+9·25
	13	—7·25	+0·75	—2·50	—11·00	—2·00	+6·50	—1·50	—2·50	+2·75	—4·00	—1·75	+0·50	—4·75	—26·75
Sum of columns	..	—15·25	—30·50	—25·50	—8·00	—14·17	+5·25	+8·50	—6·00	+13·75	+10·25	+15·50	+31·75	+25·50	+11·08

3. In reducing any considerable body of field data, it is usually convenient to adopt a suitable base number, and subtract this base number from each individual figure of the original data. In the present example, 24 oz. will be a convenient number, as this happens to be the approximate value of the general mean.* We now subtract 24 from each of the yield figures in Table I, and enter the deviation (plus or minus) from 24 in Table II. For convenience of reference, I shall use the following serial numbers for the different varieties.

No.	Variety
1.	B. × P. No. 11
2.	B. × P. No. 12
3.	B. × P. No. 15
4.	B. × P. No. 19

No.	Variety
5.	B. × P. No. 20
6.	B. × P. No. 22/1
7.	B. × P. No. 22/2
8.	B. × S. No. 23

No.	Variety
9.	B. × S. No. 24
10.	B. × S. No. 30
11.	Bhondu
12.	Parewa
13.	Surmatia

The figures in each column and in each row are next added and the sums entered as shown in Table II. (For example, the sum of the figures in row 1 is +40·50, of column 5 is —14·17, of row 6 is —0·92, and so on). Adding the marginal totals of columns we get +11·03. (This is checked by adding the marginal totals of rows.) The general mean is then given by 24+11·03/169=24·07 approximately. (This may, and should be checked by direct addition of all the yields.)

The squares of individual deviations are then taken from a table of squares (like Barlow's Tables) and entered in Table III Rows and columns are added, and then the marginal totals as before. The gross total of all (169) deviations from 24 is found to be 2590·1564.

* It does not, however, really matter what particular number is selected. For ease of subtraction, 20 might have been selected as the base number.

TABLE III.

Squares of deviations given in Table II.

		1	2	3	4	5	6	7	8	9	10	11	12	13	Total of rows
	1	20·2500	16·0000	16·0000	45·5625	2.2500	49·0000	30·2500	1·5625	81·0000	14·0625	6·2500	22·5625	30·2500	335·0000
	2	20·2500	64·0000	9·0000	33·0625	25·C000	33·0625	6·2500	1·0000	115·5625	25·0000	2·2500	33·0625	0·2500	367·7500
	3	2·2500	9·0000	4·0000	·2·2500	1·0000	14·0625	39·0625	2·2500	12·2500	5·0625	9·0000	27·5625	64·0000	191·7500
	4	1·0000	5·0625	4·0000	9·0000	3·0625	2·2500	5·0625	9·0000	14·0625	1·5625	—	0·2500	10·5625	64·8750
	5	6·2500	45·5625	20·2500	16·0000	12·2500	2·2500	3·0625	0·5625	4·0000	1·0000	20·2500	18·0625	18·0625	167·5625
	6	3·0625	—	2·2500	9·0000	13·4689	10·5625	5·0625	0·0625	25·0000	1·0000	6·2500	1·5625	9·C000	86·2814
	7	1·5625	2·2500	9·0000	16·0000	0·2560	1·0000	12·2500	7·5625	0·5625	9·0000	—	4·0000	27·5625	91·0000
	8	6·2500	36·0000	72·2500	2·2500	20·2500	9·0000	1·0000	22·5625	0·2500	0·0625	9·0000	12·2500	33·0625	224·1875
	9	6·2500	1·5625	25·0000	10·5625	1·5625	42·2560	12·2500	4·0000	0·5625	12·2500	7·5625	36·0000	6·2500	166·0625
	10	2·2500	25·0000	56·2500	1·0000	33·0625	—	4·0000	4·0000	2·2500	52·5625	25·0000	2·2500	6·2500	213·8750
	11	0·5625	10·5625	81·0000	2·2500	1·0000	0·5625	10·5625	5·0625	25·0000	12·2500	4·0000	56·2500	56·2500	265·3125
	12	10·5625	10·5625	9·0000	3·0625	3·0625	7·5625	3·0625	6·2500	30·2500	20·2500	25·0000	0·2500	3·0625	131·9375
	13	52·5625	0·5625	6·2500	121·0000	4·0000	42·2500	2·2500	6·2500	7·5625	16·0000	3·0625	0·2500	22·5625	284·5625
Total of columns.		133·0625	226·1250	314·2500	271·0000	120·2189	213·8125	134·1250	70·1250	318·3125	170·0625	117·6250	214·3125	287·1250	2590·1564

Gross (uncorrected) sum of squares = 2590·1564.

A separate table (Table IV) is now formed for the deviations for each variety, and sums of deviations obtained by addition. (For example, the sums of deviations for variety No. 9 is + 42·50). The squares of these deviations are also independently written down, and added. The total again comes to 2590·1564, thus furnishing an absolute check on the arithmetic so far.

TABLE IV.

Deviations for each variety.

(*Reference numbers of varieties on page 1.*)

	(1)	(2)	(3)	(4)	(5)	(6	(7)	(8)	(9)	(10)	(11)	(12)	(13)
1	—1·50	+0·50	+0·75	—11·00	—2·50	—1·75	—2·50	+6·50	+2·75	—4·00	—2·00	—4·75	—7·25
2	+2·50	—3·25	+1·75	—1·75	—3·00	+1·75	+1·75	+5·50	+5·00	—3·25	+4·50	+0·50	—2·75
3	0·0	+2·00	—7·50	—5·75	—1·00	+5·00	—2·50	+1·50	+7·25	+1·50	+5·00	—2·00	—1·50
4	—0·75	+1·50	+5·00	—3·25	+0·75	+7·50	+2·00	+7·50	+1·00	—2·25	+3·75	—9·00	+3·50
5	—5·00	—1·25	—2·50	—6·50	—3·50	+0·75	—3·25	+2·00	+6·00	—2·75	—1·25	—3·50	—2·50
6	—6·00	—0·50	—4·75	—1·00	+2·50	+3·50	—4·50	—0·25	+1·50	—8·50	+5·75	—3·00	—3·00
7	0·0	—1·00	+3·00	+2·00	—0·50	+3·00	—0·75	+1·50	+5·25	—3·50	—2·75	—1·25	—4·00
8	—5·00	+0·25	+1·25	+2·50	+1·00	+3·25	+1·75	+2·25	0·0	—3·00	+1·50	—3·00	—3·67
9	+4·25	+1·00	+1·75	+2·50	+4·50	+3·50	—0·75	+4·25	+4·50	+1·50	+4·00	+2·00	—6·75
10	+3·00	+2·00	+1·50	+1·25	+3·25	+3·00	+0·50	+1·75	—1·00	—2·25	+3·75	0·0	—2·25
11	—2·25	+1·50	+8·00	+1·50	+3·50	+6·25	—3·00	+3·00	+3·75	—1·50	+5·25	—1·00	—2·00
12	—5·00	+0·50	—1·50	—3·00	—8·00	—5·00	—5·75	—5·75	+1·00	—10·75	—4·50	+2·50	—5·75
13	+5·50	+2·50	+6·75	+9·00	+4·75	—4·50	+3·75	+4·00	+5·50	+1·50	+7·00	+4·00	—1·25
Total .	—10·25	+5·75	+13·50	—13·50	+1·75	+26·25	—13·25	+33·75	+42·50	—34·25	+30·00	—31·50	—39·17

The sums of deviations for rows, columns, and varieties are next written in Table V, columns 2, 4, and 6 respectively. (It will be noticed that the sum of deviation of row 3 is + 26·00 which is taken from Table II, or the sum of deviation of variety No. 9 is + 42·50 which is taken from Table IV, and so on). Squares of these sums of deviations are then entered in columns 3, 5, and 7 respectively. Adding the figures in columns 3, 5, and 7 we find the sums of squared deviations to be 8312·7214, 4406·3514, and 8885·4764 respectively.

TABLE V.

Sums of deviations for rows, columns, and varieties.

No.	Rows		Columns		Varieties	
	(1) Deviation	(2) Squares	(3) Deviation	(4) Squares	(5) Deviation	(6) Squares
1	+40·50	1,640·2500	—15·25	232·5625	—10·25	105·0625
2	—56·00	3,136·0000	—30·50	930·2500	+ 5·75	33·0625
3	+26·00	676·0000	—25·50	650·2500	+13·50	182·2500
4	+14·50	210·2500	— 8·00	64·0000	—13·50	182·2500
5	+26·25	689·0625	—14·17	200·7889	+ 1·75	3·0625
6	— 0·92	0·8464	+ 5·25	27·5625	+26·25	689·0625
7	+ 1·00	1·0000	+ 8·50	72·2500	—13·25	175·5625
8	—18·25	333·0625	— 6·00	36·0000	+33·75	1,139·0625
9	—23·25	540·5625	+13·75	189·0625	+42·50	1,806·2500
10	+ 2·00	4·0000	+10·25	105·0625	—34·25	1,173·0625
11	+16·75	280·5625	+15·50	240·2500	+29·50	870·2500
12	+ 9·25	85·5625	+31·75	1,008·0625	—31·50	992·2500
13	—26·75	715·5625	+25·50	650·2500	—39·17	1,534·2889

As we have worked with sums of 13 individual figures, in the case of rows, columns and varieties these three sums are divided by 13, giving the following results.

GROSS SUM OF SQUARES.

Rows	639·4401
Columns	338·9501
Varieties	683·4982

So far the sum of squares have been found for the deviations of each plot yield from the arbitrary base number 24. We require however the sums of squares for deviations from the true mean (24·07). These can be obtained by applying a correction to the sum of squares obtained above.

The sum of all deviations with respect to base number 24 is + 11·08. Squaring 11·08 we get 122·7664. Dividing this number by 169 (the total number of all deviations) we get —0·7264, the required correction. This must be subtracted, from the above uncorrected sums of squares of deviations.*

Hence, we derive the corrected sum of squares.

	Sum of squares
Rows	638·7137
Columns	338·2237
Varieties	682·7718

4. We are now in a position to form Fisher's Table of Analysis of Variance (Table VI). In column 1 is given the nature of variation. In column 2 the corresponding number of degrees of freedom. These represent the total possible number of independent comparisons in each case. For example, for the 13 rows only 12 independent comparisons are possible, and similarly for the columns and varieties. For the whole sample of 169 plots the total number of comparisons possible is 168. (In fact in field trials, the number of degrees of freedom is usually obtained by subtracting 1 from the size of the sample).

The sums of squares of deviations for rows, columns and varieties 638·7137, 338·2237 and 682·7718 are written down in column 3, and added giving a total of 1659·7092. This represents the contribution of 36 degrees of freedom (usually written as D. F.) due to rows *plus* columns *plus* varieties (each of which absorbs 12 degrees of freedom). The total sums of squares of deviations, 2589·4300, is obtained by applying the correction (— 0·7264) to 2590·1564, the gross total already found in Table III. This represents 168 degrees of freedom. Subtracting 1659·7092 from 2589·4300, we get finally 929·7208 as the sums of squares of deviations due to the residual causes of variation (usually called random errors) with 168—32=136 degrees of freedom. For purposes of comparison it is this residual variation which gives the probable margin of experimental errors (including residual effects of soil heterogeneity). The enormous advantage of eliminating the effects of other factors of variation is obvious.

5. Dividing the sums of squares (column 3) by the corresponding degrees of freedom (column 2), we get the quantities known as "variances" given in column 4. We notice that the variance due to differences of varieties is 56·8976, while the random variance is 7·0433. (For purposes of comparison only these two variances

* This correction is always negative, *i.e.*, must always be subtracted.

are required in practice). We may now use Fisher's 'z-test' to find whether the varietal differences may be considered significant in comparison with the random variance, *i.e.*, whether 56·8976 may be considered to be significantly greater than 7·0433. The natural logarithms (that is logarithms to be base 'e', and not to the base "10") of the variances are required for this purpose. They are entered (from mathematical tables) in column 5 of Table VI.

TABLE VI.

Analysis of variance.

Variance due to	D. F.	Sum of squares	Mean square	$\log_e(6^2)$.
Varieties . .	12	682·7718	56·8976	4·04112
Columns . .	12	338·2237	28·1853	..
Rows . .	12	638·7137	53·2261	..
Error . .	132	929·7208	7·0433	1·95203
Total .	168	2589·4300	..	2·08909

The z-test may be now applied in the following way. Let v_1 and v_2 be the variances for varietal differences and random errors respectively.

Then $z=\frac{1}{2}(\log_e v_1 - \log_e v_2)=1·0445$ with n_1 (the D. F. corresponding to the larger variance)=12, and n_2=132. We may now use Fisher's Table VI (Statistical Methods, page 215). We find that the one per cent. point (that is, for probability of 1 in 100), with n_1=12, and n_2=60, $z=0·4574$, while for n_1=12, and $n_2=\infty$, $z=0·3908$. The observed value of z=1·0445. It is clear that the odds are much greater than 100 to 1 against such a value of z (=1·0445) occurring by chance. We conclude that the observed value of z=1·0445 indicates a significant difference between 56·8976 and 7·0433. That is, we conclude that the varietal differences are statistically significant in comparison with the random error of the experiment.

6. We may now proceed to test the individual differences in yield between the different varieties. The residual variance is 7·0433. The variance for mean values based on samples of 13 will be given by 7·0433/13. The variance of differences between any two such mean values (each based on samples of 13) is given by $2 \times 7·0433/13=1·08374$. Extracting the square root of this quantity we obtain 1·041 as the value of the standard error for comparison of mean yields based on 13 replications each.

The differences in mean yield are tabulated systematically in Table VII.

TABLE VII.

Differences in yield.

No.	Variety.	B.×P. No. 11.	B.×P. No. 12.	B.×P. No. 15.	B.×P. No. 19.	B.×P. No. 20.	B.×P. No. 22/1.	B.×P. No. 22/2.	B.×S. No. 23.	B.×S. No. 24.	B.×S. No. 30	Bhondu	Parewa.	Surmatia.	(16) Difference for (24·07).	(17) Mean yield of each variety Mean yield.
1	B.×P. No. 11	—	+1·23	+1·83	—0·25	+0·92	+2·81	—0·23	+3·38	+4·06	—1·85	+3·05	—1·63	—2·22	—0·86	23·21
2	B.×P. „ 12	—1·23	—	+0·60	—1·48	—0·31	+1·58	—1·46	+2·15	+2·83	—3·08	+1·82	—2·86	—3·45	+0·37	24·44
3	B.×P. „ 15	—1·83	—0·60	—	—2·08	—0·91	+0·98	—2·06	+1·55	+2·23	—3·68	+1·22	—3·46	—4·05	+0·97	25·04
4	B.×P. „ 19	+0·25	+1·48	+2·08	—	+1·17	+3·06	+0·02	+3·63	+4·31	—1·60	+3·30	—1·38	—1·97	—1·11	22·96
5	B.×P. „ 20	—0·92	+0·31	+0·91	—1·17	—	+1·89	—1·15	+2·46	+3·14	—2·77	+2·13	—2·55	—3·14	+0·06	24·13
6	B.×P. „ 22/1	—2·81	—1·58	—0·98	—3·06	—1·89	—	—3·04	+0·57	+1·25	—4·66	+0·24	—4·44	—5·03	+1·95	26·02
7	B.×P. „ 22/2	+0·23	+1·46	+2·06	—0·02	+1·15	+3·04	—	+3·61	+4·29	—1·62	+3·28	—1·40	—1·99	—1·09	22·98
8	B.×S. „ 23	—3·38	—2·15	—1·55	—3·63	—2·46	—0·57	—3·61	—	+0·08	—5·23	—0·33	—5·01	—5·60	+2·52	26·59
9	B.×S. „ 24	—4·06	—2·83	—2·23	—4·31	—3·14	—1·25	—4·29	—0·08	—	—5·91	—1·01	—5·09	—6·28	+3·20	27·27
10	B.×S. „ 30	+1·85	+3·08	+3·68	+1·60	+2·77	+4·66	+1·62	+5·23	+5·91	—	+4·91	+0·22	—0·37	—2·71	21·36
11	Bhondu .	—3·05	—1·82	—1·22	—3·30	—2·13	—0·24	—3·28	+0·33	+1·01	—4·90	—	—4·68	—5·27	+2·19	26·26
12	Parewa .	+1·63	+2·86	+3·46	+1·38	+2·55	+4·44	+1·40	+5·01	+5·69	—0·22	+4·68	—	—0·59	—2·49	21·58
13	Surmatia .	+2·22	+3·45	+4·05	+1·97	+3·14	+5·03	+1·90	+5·60	+6·28	+0·37	+5·27	+0·59	—	—3·08	20·90
	Mean yield .	23·21	24·44	25·04	22·96	24·13	26·02	22·98	26·59	27·27	21·36	26·26	21·58	20·90	—	—

N. B.—Column (16) gives the difference in yield of each variety from the general mean yield (24·07 oz.)
Column (17) gives the mean yield of each variety. These figures are also repeated in the last row.

In order to appreciate the significance of these differences we proceed in the following way. We have seen that the standard error of the mean difference is 1·041. We may use Fisher's t-table (Table IV, p. 139), although in this case the classical theory will give practically correct results. Adopting a level of significance of 1 per cent. (P=·01, or odds of 100 to 1), we find that for n=132, the critical value of "t" is 2·536. Since "t" is expressed in terms of the standard error, we must multiply it by 1·041, and obtain the critical value of the difference to be 2·640 in the present case. In other words the odds are 100 to 1 that any observed difference (in mean yields given in Table VII) of this magnitude is definitely significant *. For any pair of varieties we can therefore find the significance of the comparison by a mere inspection of Table VII. For example for variety No. 9 (*i.e.*, B. × S. No. 24) we notice that it gives a significantly better yield than No. 1 (B. × P. No. 11), No. 4 (B. × P. No. 19), No. 5 (B. × P. No. 20) No. 7 (B. × P. No. 22/2), No. 10 (B. × S. No. 30), No. 12 (Parewa) and No. 13 (Surmatia). Or let us compare No. 2 (B. × P. No. 12) and No. 6 (B. × P. No. 22/1). The observed difference is 1·58, and is not significant. We cannot assert that on a repitition of the trial 'B. × P. No. 22/1' is definitely more likely to produce a better yield than 'B. × P. No. 12'.

Expressing the critical difference 2·911 as a percentage of the general mean yield (24·07), we get 11·0 per cent. approximately. The order of accuracy attained in the present experiment is therefore such that differences in yield of the order of 11 per cent. may be detected with a certainty of 100 to 1. With a lower level of significance (of 20 to 1), the critical difference is 8·4 or 8 per cent. approximately.

7. If we compare the results given by the author (pp. 370—371) with my results, we find several discrepancies. The authors state that the difference between 'B. × P. No. 11' and 'Bhondu' is insignificant, my analysis shows that the observed difference of 3·05 may be considered to be significant. In the same way I find that the difference between 'B. × P. No. 19' and 'Bhondu' (3·30), is also significant. Adopting the 5 per cent. level of significance (for which the critical difference is 2·022), we notice, that two other differences ('B. × P. No. 20' and 'Bhondu', 'B. × P. No. 20' and 'Parewa') rejected as insignificant by the authors may also be considered significant.

The authors give 20 comparisons, out of which 10 are insignificant. Against this in Table VII we have figures for all possible comparisons, in this case 68 in all. With 1 per cent. probability, (critical difference 2·64), no less than 34 differences out of 68 are significant. With 5 per cent. probability (critical difference 2·02)

* If we use a lower level of significances of 5 per cent., the critical value of 't' is 1·942, and the critical difference is 2·022.

8 more differences may be considered significant. We find, therefore, that on the basis of the present experiment 42 differences are significant, while 26 must be considered insignificant.

The above discussion shows that the present experiments are actually more valuable than one would gather from the analysis originally given by the authors.

The advantages of the systematic procedure described above is then two-fold :—

(*a*) There is a substantial gain in the precision of the comparison, and

(*b*) the process is exhaustive, so that not a single significant difference is likely to be missed.

Further, as already pointed out, the statistical theory used is one which was specially developed to meet the requirements of small samples.

The numerical calculations were completed under the supervision of my computing assistant Babu Sudhir Kumar Banerjee, and were rendered possible by a research grant from the Imperial Council of Agricultural Research.

SELECTED ARTICLES

CHEMICAL METHODS FOR ESTIMATING THE AVAILABILITY OF SOIL PHOSPHATE

BY

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The purpose of this paper is to record the main points of interest developed by the writer during the past three years of an investigation of chemical methods for determining the availability to plants of soil phosphate. Since the days of Liebig this has been a live question, and it still lacks a satisfactory answer.

Available soil phosphate is, for the present purpose, defined as "that part of the soil phosphate which may be absorbed or used by ordinary crop plants in the production of plant substances." Also it is assumed that different species of plants may have quite unlike power to obtain an adequate supply of phosphate from a given soil which is deficient in available phosphate.

In the endeavor to find a somewhat satisfactory procedure, several methods proposed by others have been examined (the results are reported at the end of this paper), much experimental work has been done, and some new methods have been worked out. It is thought that some of the new methods permit a closer approach to an adequate solution of the problem than any previously published chemical methods.

Introduction.

A satisfactory estimation of the availablity of soil phosphate by chemical methods may be difficult or impossible for several reasons. Some are here mentioned.

Phosphorus occurs in the soil in several forms of combination, both mineral and organic. This paper is concerned with mineral phosphate only. Apatite, $Ca_3(PO_4)_2CaF_2$; chlorapatite, $Ca_3(PO_4)_2CaCl_2$; hydroxyapatite, $[Ca_3(PO_4)_2]_3Ca(OH)_2$; wagnerite, magnesium phosphate; and wavellite, aluminum phosphate, are soil minerals. According to Russell (21), hydroxyapatite is probably the chief source of

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soil phosphate. These minerals are relatively insoluble in water. Since most mineral phosphates are very insoluble in water, there is but little PO_4 dissolved in the soil solution at any one time. The amount of PO_4 in solution is increased by CO_2 or other acids, provided they do not at the same time increase too much the concentration of those cations which tend so repress the dissolving of phosphate.

Some students believe that plants have power to absorb certain nutrients from the solid state, without first having to be dissolved in the soil solution. This is said to be true of phosphates. Also plants have the power of selection so that they can take up PO_4 without at the same time absorbing the cations combined with it.

Another difficulty in estimating the availability to plants of soil phosphate by chemical means is found in the fixation of PO_4 by the soil so that it becomes insoluble in the soil solution and largely unavailable to plants. The determination of whether fixation is a purely chemical phenomenon or is more or less dependent on surface action and colloidal effects has not been made a part of this study. It seems probable that the availability of fixed phosphate depends much on the form in which it is fixed particularly as to whether it is combined with Fe, Al, or Ca, but chemical agents do not well separate these different phosphates.

Even though the supply of available phosphate is adequate, plants may fail on account of the lack of some other nutrient or perhaps from the presence of some unknown or unsuspected injurious or toxic substance or condition, but such adversities to plants will have little effect on the solubility of soil phosphate in chemical reagents. Since chemical methods of estimating the availability of phosphate depend on first getting it into solution, and since chemical solubility may be very little dependent on several of the aforementioned factors, it is evident that chemical methods can not well be expected to imitate the action of plants in estimating the availability to plants of soil phosphate.

After plants have grown upon a soil which has been treated with phosphate, it seems unreasonable to expect that any chemical test applied to the cropped soil give a proper representation of the soil as the plant had to deal with it at the start immediately after the phosphate was added, particularly if the phosphate was mostly water-soluble at first.

EXPERIMENTAL.

To enable the reader to understand better the experimental work recorded in the following, there is presented on page 172 a tabular statement giving the laboratory number, name, response to phosphate fertilizers, and general fertility of the soils most used in this work.

The methods used in obtaining soil extracts for this study are of two kinds: equilibrium methods, in which the soil is shaken with a volume of solution for some

time, then the filtered solution is analysed; and percolation methods, by which the soil is percolated with a solvent to more or less complete removal of PO_4 or other ions, then the percolate is examined. The numerical results obtained by these two procedures are usually unlike and not properly comparable. It is not intended to convey the idea that complete equilibrium is attained in the first case, but only a near approach to it; or that complete extraction, only approximate, is obtained by percolation. It is thought that in either case the process was carried far enough to show the value of the procedure.

Character of the soils studied.

Number	Name	Response to PO_4 fertilizer	General fertility
1C	Yolo silty clay loam	Little	Very good
30	Fresno fine sandy loam	None	Very good
36	Farwell sandy loam	Large	Low
37	Nord sandy loam	Large	Low
38	Vina silty clay loam	Little	Good
40	Altamont-Olympic loam, wash	None	Very high
45	Aledo, Ill., clay loam and rock phosphate	...	Good
46	Aledo., Ill. Same untreated	.....	Poor
53	Delhi sand	Some	Fair
59	Aiken clay	Very great	Medium
64	Vina silt loam	Great	Poor
65	Yolo loam	Little	Very high
66	Aiken clay	Great	Medium
68	Tejunga fine sand	Little	Good
69	Sites clay loam	Great	Medium
78	Fine sandy loam	Great	Poor
80	Handford fine sandy loam	Great	Poor

Equilibrium methods for giving an idea of the available PO_4 in a soil are subject to the general criticism that the effects produced in this way are very unlike those produced by a plant drawing nutrients from the soil in a selective manner. In an equilibrium extraction, some of the products of the action of weak solvents may tend to repress solubility of PO_4, *e.g.* Ca and Mg brought into solution by weak or dilute acids. This has been pointed out by Teakle (23) and others.

It seems irrational to apply such concentrated acids as have commonly been used, such as strong HCl; 0·2*N* HNO_3, pH 0·7; or 1 per cent citric, pH 2·1; since plants have no such strong solvent agents at their disposal.

Perhaps the best reason for using equilibrium methods is that they may supply useful indications with the least expense of time and labor. A fair idea of the power of a soil to supply PO_4 may be quickly had in this way, but at the same time the result may give an erroneous notion of the availability to plants of the PO_4 thus extracted.

Equilibrium methods.

Ratio of soil to solution 1:5.—Usually 40 gm. of soil was placed in a 400-cc. bottle with 200 cc. of solution and agitated in an end over end shaker for three

hours or more. The mixture was filtered on Whatman No. 12, 24 cm. folded filters, and after nearly all the liquid had run through, the solution was analysed. PO_4 was determined by the molybdenum blue method about as described by Parker and Fudge (19), pH by color comparison, Ca by turbidity after adding $(NH_4)_2O_4$, Fe by color comparison after adding $K_4Fe(CN)_6$ and HCl.

A single extraction of any soil by any solvent which changes the pH materially gives only a very incomplete picture of the character of the soil in regard to its power to supply phosphate under changing conditions. But by using three or four different concentrations of a dilute acid, much more is learned. In this, it is important to note the pH of the solution removed from the soil. This presumes that only dilute acids are used, so that the pH of the extract is never brought below 2.

In this manner, more than 50 different soils have been examined. A few typical results are presented in Table 1, which gives the figures obtained with citric, oxalic, and hydrochloric acids of 0·005, 0·025, 0·050, and 0·100 normalities. This shows the great differences between different soils and also the different results given by the different acids of the same concentration on any one soil. These figures indicate something of the buffer power of the soil, or its power to neutralize acid, how much acid is needed to reduce the pH of the soil to some lower point, and how much PO_4 may be brought into solution at that pH. It is apparent that the amount of PO_4 dissolved is closely related to the pH of the solution when simple acids such as HCl or HNO_3 and probably also CO_2, are used. Citric and oxalic acids form soluble complexes with Fe † † †, and other cations, so have greater solvent effect than their concentration of hydrogen ion should give of itself. Oxalic acid is peculiar in that it removes Ca from solution at pH above 3 to 4, thus permitting more PO_4 to remain in solution than would HCl, H_2SO_4, or HNO_3. Also, citric, and oxalic acids are not appropriate because they must be removed from the solution before it is tested for PO_4 content by the molybdenum blue method. Carbon dioxide equilibrium at ordinary atmospheric pressure has too small a concentration of hydrogen ion to be an active solvent. But when used continuously as in percolation with water saturated with CO_2, it has great solvent effect since the products of reaction are continually removed, thus allowing the reaction to proceed to completion in one direction. This largely accounts for the great effects on the soil produced by rain water.

Buffer power of the soil in relation to the solution of soil phosphates by dilute acids.—The figures appearing in Table 1 show how different is the effect of a dilute acid on different soils. It seems evident that instead of using the same arbitrary concentration of acid on all soils, an amount of acid should be used such that the pH of the extract will be the same for all soils. If this is accomplished the results should be properly comparable. The figures given in Table 1 may be plotted so

that by interpolation or extrapolation the PO_4 soluble at any definite pH, such as 4, may be inferred. Figure 1 gives such graphs for a few soils, and Table 2 gives PO_4 at pH 4 for several soils. These figures seem to give a much better idea of the phosphate-supplying power of the soil than do those found by the use of the same amount of acid for all soils, in which case the pH of the extracts often differs greatly.

There are two objections to this method. Unless two points somewhere near pH 4 happen to be hit in the determinations, the figure inferred for pH 4 may be wide of the truth, and this method requires the preparation and analysis of three or four solutions. To avoid these objections, it was sought to make the extraction by the use of such an amount of acid that the extract would have a pH of approximately 4. This was found difficult to accomplish in a simple practical way because when a soil is mixed with a dilute, highly ionized acid, the pH does not remain at one point for more than a few minutes, and because it is necessary to make some preliminary experiments to indicate about how much acid will be needed to bring the pH of the soil to any definite point.

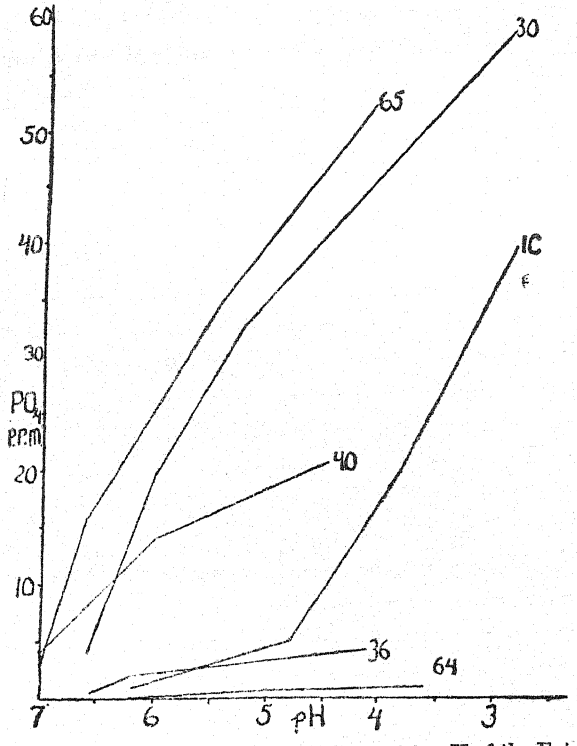


Fig. 1. Relation of PO_4 in Dilute Acid Extract to pH of the Extract.

The figures given in Table 3 show how rapidly the solution changes after acid has been added and how greatly soils differ in this respect when the highly ionized, slightly buffered HCl is used. Addition of KCl, suggested by Truog (24), to the acid did not give enough buffer effect to be of any advantage. When citric acid was used, its buffer power made the change in content of PO_4 of the solution much slower, but it is undesirable for reasons stated on page 440. Acetic acid is little ionized, highly buffered, and otherwise an appropriate solvent. Although the PO_4 content of the extract made with this acid is less constant in some cases than when citric acid is used, it seems better adapted for estimating available PO_4 than any of the other common acids. Oxalic acid, suggested by Vanstone (25), is even less appropriate than citric acid. Some of the results obtained with acetic acid are given in Table 4.

TABLE 1.

PO_4 and pH in 1 : 5 equilibrium extracts of soils with citric, oxalic, and hydrochloric acids of four different concentrations.

Soil number	Acid	0·005 *N* Acid		0·025 *N* Acid		0·05 *N* Acid		0·1 *N* Acid	
		PO_4 p.p.m.	*pH*	*PO_4* p.p.m.	*pH*	*PO_4* p.p.m.	*pH*	*PO_4* p.p.m.	*pH*
10	Citric	5	5·2	16	4·2	27	3·6	44	3·0
	Oxalic	10	5·2	27	4·4	50	3·3	66	2·4
	HCl	5	4·8	20	3·8	40	2·8	40	1·5
30	Citric	24	4·2	33	3·3	40	2·9	57	2·6
	Oxalic	40	3·9	66	2·4	80	1·8	80	1·4
	HCl	40	4·8	80	2·8	80	1·5	80	1·5
65	Citric	40	5·2	50	4·2	66	3·6	66	3·0
	Oxalic	40	5·3	44	4·0	56	3·0	80	2·0
	HCl	40	5·2	56	3·0	80	2·0	80	1·5
38	Citric	..	..	10·0	4·4	13	3·7	20	3·0
	Oxalic	..	..	8·0	4·8	20	3·6	50	2·4
	HCl	..	..	3·2	4·0	6·6	2·4	10	1·4
64	Citric	..	..	8·8	4·5	13	3·6	22	3·0
	Oxalic	..	..	8·0	5·0	20	3·6	66	2·4
	HCl	..	..	7·2	3·6	10	2·2	13	1·5

TABLE 1—*contd.*

PO_4 and pH in 1 : 5 equilibrium extracts of soils with citric, oxalic, and hydrochloric acids of four different concentrations—contd.

Soil Number	Acid	0·005 *N* Acid		0·025 *N* Acid		0·05 *N* Acid		0·1 *N* Acid	
		PO_4 p.p.m.	*pH*	*PO_4 p.p.m.*	*pH*	*PO_4 p.p.m.*	*pH*	*PO_4 p.p.m.*	*pH*
35	Citric	..	..	5·6	3·8	9·0	3·2	11·0	2·8
	Oxalic	..	..	2·0	4·4	5·0	3·4	33·0	2·4
	HCl	..	..	0·8	3·2	1·2	2·0	2·4	1·4
59	Citric	0·2	5·2	0·4	3·9	0·3	3·2	0·3	2·5
	Oxalic	0·2	5·0	1·0	4·4	0·4	3·4	1·6	2·5
	HCl	0·1	4·2	0·1	3·0	0·1	2·0	0·1	1·0
37	Citric	1·0	7·5	1·2	7·3	1·6	7·0	10·0	4·4
	Oxalic	1·0	7·5	1·2	7·3	3·2	6·8	11·4	4·5
	HCl	0·6	7·5	1·2	7·0	3·2	5·6	11·4	4·0
36	Citric	..	..	13	4·4	20	3·6	26	3·0
	Oxalic	..	..	16	4·8	50	3·6	80	2·4
	HCl	..	..	8	4·0	20	2·2	40	1·8

Probably a much larger proportion of solvent to soil would make it easier to hold the pH of the extract to some definite point. In Table 5 are shown some of the results obtained with 2 gm. of soil to 200 cc. of solution. Although the concentration of PO_4 in the extract is much higher in the 1:5 extracts, the amount dissolved from the soils is much greater in the 1:100 extracts. The greater proportion of water dissolves much more PO_4 from the soil. In the more dilute extracts the relative supplying power of the different soils is quite different from that found by the 1 : 5 extraction. But the latter represents much better the actual phosphate-supplying power of the soils as indicated by growth of plants on them.

This brings out the point that in most soils only a small proportion of the phosphate present dissolves in weak solvents at small dilutions. It sums reasonable to

think that the nearer the ratio of soil to solution is to the ratio existing in a soil where plants are growing. the nearer will the results for PO_4 represent the condition

TABLE 2.

PO_4 in 1:5 equilibrium extract of soils with dilute HCl, at pH 4 by interpolation.

Fertility of soil	Soil number	PO_4 at pH 4
		p.p.m.
Very fertile	1C	17
	30	44
	40	23
	65	52
Good	36	4·0
	37	11·0
	38	1·5
	68	14·0
Aledo, Ill., and rock phosphate	45	13·0
Aledo, Ill., not fertilized	46	1·4
Very poor	64	1·0
Exceedingly poor	59	0·1
	66	0·1
	69	0·4
	76	0·2

with which the plant has to deal. The work of others, as well as some done during this study, shows that the concentration of PO_4 in water extracts of some soils is almost the same regardless of the ratio of soil to solvent until large dilutions are reached. Results obtained in this study are shown in Table 6.

The same thing is shown in Table 7, giving the results from extracting several soils with dilute acetic acid with four different ratios of soil to solvent. In this experiment, soils 64, 38, 89B, and 91A gave somewhat the same concentration of PO_4 in the solution whether the ratio of soil to solution was 1/5 or 1/40.

Soils 1C, 30, 36, 68, 75 and 80 show increasing amounts of PO_4 extracted from the soil as the ratio of solvent to soil is increased, yet the increase in PO_4 is not equal to the increase in dilution. In Table 5 are some figures found by a dilution of 1/100,

and the other extreme is shown in Table 8, with a ratio of 2/1. Pot cultures show that the PO_4-supplying power of the soil is much better represented by the figures

TABLE 3.

Change in pH and PO_4 with change in time after acid has been added.

120 gm. soil shaken with 600 cc. of solution.

Soil number	0.1 *N* HCl added	Time for reaction	Solution after filtering	
			pH	PO_4
	c. c.			*p.p.m.*
10	300	10 minutes	2·05	50
		1 hour	2·21	50
		5 hours	2·62	29
		24 hours	3·13	10
		72 hours	3·38	4
30	120	10 minutes	2·05	40
		1 hour	2·17	66
		5 hours	2·31	100
		24 hours	2·53	111
		72 hours	2·70	117
35	200	10 minutes	2·07	0·88
		1 hour	2·57	0·58
		5 hours	3·19	0·40
		24 hours	3·54	0·28
		72 hours	3·54	0·20
36	300	10 minutes	2·09	25·0
		1 hour	2·39	25·0
		5 hours	2·66	15·0
		24 hours	3·00	5·0
		72 hours	3·21	2·4

TABLE 3—*contd.*

Change in pH and PO_4 with change in time after acid has been added—contd.

120 gm. soil shaken with 600cc. of solution—*contd.*

Soil number	0·1 *N* HCl added	Time for reaction	Solution after filtering	
			pH	PO
	cc.			*p.p.m.*
38	170	10 minutes	2·79	4·8
		1 hour	3·17	2·8
		5 hours	3·73	1·7
		24 hours	4·09	0·8
		72 hours	4·05	0·7
64	270	10 minutes	2·01	11·4
		1 hour	2·31	8·8
		5 hours	2·55	4·8
		24 hours	2·87	1·6
		72 hours	3·12	1·1

given in Table 7. The chief reason for using a 1:5 ratio instead of 2:1 of soil and solvent, is that it is much more convenient, and still the dilution is not so great that the results are not applicable.

TABLE 4.

Change in pH and PO_4 with time after acid has been added.

80 gm. soil shaken with 400 cc. solution.

Soil number	Time for reaction	0·1 *N* citric acid	pH	PO_4 in solution	0·1 *N* acetic acid	pH	PO_4 in solution
		cc.		*p. p. m.*	*cc.*		*p. p. m.*
1C	5 minutes	70	4·13	8·0	250	..	..
	75 minutes		4·29	11·4		4·04	12
	5 hours		4·46	11·4		4·04	10
	16 hours		4·58	11·4		4·09	8
	48 hours		4·90	11·4		..	..

TABLE 4—*contd.*

Change in pH and PO_4 with time after acid has been added—contd.

80 gm. soil shaken with 400 cc. solution—*contd.*

Soil number	Time for reaction	0·1 *N* citric acid	pH	PO_4 in solution	0·1 *N* acetic acid	pH	PO_4 in solution
		cc.		*p. p. m.*			*p. p. m.*
36	5 minutes	70	4·18	3·4	270	..	..
	75 minutes		4·32	6·6		4·01	6·4
	5 hours		4·40	6·6		3·99	4·4
	16 hours		4·51	6·6		4·05	2·8
	48 hours		4·70	6·6		..	..
38	5 minutes	70	4·13	1·4	220	..	..
	75 minutes		4·32	2·3		4·05	1·44
	5 hours		4·51	2·4		4·09	1·24
	16 hours		4·74	2·4		4·13	1·14
	48 hours		5·17	1·8		..	..
30	1 hour		..	..	100	3·97	19
	5 hours		..	..		3·97	22
	24 hours		..	..		4·05	25
64	5 minutes		..	..	220	3·96	3·2
	1 hour		..	..		4·05	2·0
	5 hours		..	..		4·03	1·2

TABLE 5.

PO_4 extracted from soils by dilute acetic acid at approximately pH 4.

2 gm. soil to 200 cc. solution shaken 1 hour.

Soil	0·1 *N* H Ac in 200 cc.	pH	PO_4 in solution
	cc.		*p. p. m.*
1C	10	4·1	4·0
30	5	4·1	2·4
36	10	4·1	2·4
37	20	4·2	2·9
23	10	4·0	1·2
89B	5	4·0	1·2

The important points in the preceding discussion may now be summed up to show what facts and conditions should be considered in devising a method for

TABLE 6.

PO_4 in equilibrium extracts of soils with several different proportions of ordinary distilled water.

Soil number	Ratio of soil to water									
	1/1	1/2	1/4	1/10	1/20	1/50	1/100	1/500	1/1,000	1/2,500
	PO_4 p.p.m. in solution									
1C	0·20	..	0·20	..	0·20	..	0·16	0·20	0·12	0·02
30	4·40	..	3·60	..	2·00	..	0·70	0·16	0·02	0
36	..	0·50	..	0·40	..	0·24	0·20	0·16	0·08	0
37	..	0·50	..	0·60	..	0·28	0·12	0·10	0·04	0
38	0·32	..	0·40	..	0·12	..	0·20	0·12	..	0·06
35	..	..	0·04	..	0·04	..	0·06	0·10	..	..

TABLE 7.

PO_4 in acetic acid extracts of soils with various weights of soil to 200 cc. of solvent mixture, shaken one hour before filtering.

Soil number	Soil	0·1 *N* H Ac	pH	PO_4 in solution	Soil number	Soil	0·1 *N* H Ac	pH	PO_4 in solution
	gm.	*cc.*		*p.p.m.*		*gm.*	*cc.*		*p.p.m.*
1C	5	30	3·97	6·8	64	5	29	3·92	3·1
	10	46	3·97	8·8		10	43	3·94	2·7
	20	77	3·95	11·4		20	70	3·92	2·2
	40	140	3·95	13·2		40	125	3·90	2·2
30	5	21	3·85	6·0	38	5	35	3·94	2·0
	10	28	3·83	9·0		10	50	3·96	2·0
	20	40	3·85	14·5		20	85	3·92	2·0
	40	65	3·85	25·0		40	150	3·92	2·0

TABLE 7—*contd.*

PO_4 in acetic acid extracts of soils with various weights of soil to 200 cc. of solvent mixture, shaken one hour before filtering—contd.

Soil number	Soil	0·1 *N* H Ac	pH	PO_4 in solution	Soil number	Soil	0·1 *N* H Ac	pH	PO_4 in solution
	gm.	*cc.*		*p.p.m.*		*gm.*	*cc.*		*p.p.m.*
36	5	30	3·96	3·6	89B	5	20	3·85	1·4
	10	46	3·96	4·8		10	25	3·73	1·8
	20	80	3·90	6·2		20	30	3·97	1·2
	40	145	3·92	8·0		40	40	4·01	1·1
80	5	30	4·16	6·0	91A	5	20	3·99	1·4
	10	45	4·23	9·0		10	27	4·07	1·2
	20	75	4·25	14·5		20	42	4·01	1·0
	40	140	4·29	20·0		40	70	3·97	1·0
68	5	25	3·75	3·0	92A	5	23	4·01	4·4
	10	35	3·71	4·4		10	30	4·05	3·6
	20	55	3·67	5·4		20	45	4·03	2·7
	40	95	3·65	7·2		40	73	4·05	1·5
75	5	30	3·90	12·0		..	..	..	..
	10	45	3·96	16·0		..	..	..	..
	20	75	3·92	27·0		..	..	..	..
	40	140	3·90	40·0		..	..	..	..

determining availabilty of soil phosphates by equilibrium extracts and to give results numerically properly comparable:—

Soils differ in power to neutralize acids, "buffer power." Acids bring into solution cations which tend to remove PO_4 from solution between pH 3 and 8.

Chemical reagents have not the selective power of plants, and plants have no strong solvent powers similar to strong acids.

The best kind and strength of acid for estimating the availability of soil phosphates are not known, but it should not be a strong, highly ionized, slightly buffered acid, since such will not hold the pH or PO_4 constant for more than a few minutes.

A highly buffered acid makes it much easier to hold a constant pH and approximately constant PO_4, though in any case PO_4 in solution changes even if pH is held constant, but citric and oxalic

acids are not appropriate because they form soluble complexes with Ca, Mg, Al, and Fe, thus preventing them from having their normal effect on solubility of PO_4 at the corresponding pH.

TABLE 8.

Equilibrium extracts.

200 gm. soil with 100 cc. dilute acid.

Soil number	0·1 *N* HCl used	pH of extract	PO_4 in extract	Time allowed for reaction
	c.c.		*p.p.m.*	*hours*
10	60	4·0	1·6	15
30	30	4·0	40·0	4
35	50	3·7	0·2	4
36	100	4·4	1·3	15
37	60*	5·2	2·6	15
38	55	4·8	0·4	15
64	75	4·2	0·3	15
76	30	3·4	0·8	4
79	50	3·8	0·2	4

* *N* HCl.

Acetic acid has not these defects, is well buffered, and is convenient.

To be properly comparable, all extracts should have approximately the same pH, say 4, and all other conditions of making the extracts, such as time, volume of solution, and method of shaking, should be similar.

Time required to reach equilibrium is not known but if 24 hours be taken arbitrarily, it will be sufficient for most soils and the results will be comparable for all.

The amount of acid needed for any particular soil must be found by experiment.

Most previous investigators have not controlled these conditions adequately in order to obtain properly comparable results.

To avoid these difficulties as much as possible, the following described procedure is proposed as being practical, easily executed, not very time-consuming or expensive, and as giving results properly comparable, as well as may be expected of any empirical procedure.

Availability test for soil phosphate. A preliminary test is made to learn approximately how much acid is needed to bring the soil to pH 4. Place 10 gm. of soil in a 100-cc. bottle with 10 to 20 cc. of water. Close the bottle with a soft rubber stopper and shake vigorously a few seconds. Remove the stopper and press the end against a white porcelain plate, making a mud spot. Place a drop of suitable

indicator on the mud spot. This gives a good indication of the pH of the soil. Now add a little 0·1 *N* HAc to the mud in the bottle, shake again, make another mud spot, and determine the pH. In this way, continue until a pH of 4 is obtained. This requires only a few minutes. Next calculate how much acid will be needed to bring 40 gm. of soil to pH 4. To each of three 300-cc. bottles add 40 gm. of soil and 200 cc. of dilute acid. One bottle should have about half as much acid as is calculated to bring the soil to pH 4, one bottle should contain the estimated amount and the other considerably more than that amount of acid, but the total liquid should be the same in all three.

Shake the bottles in an end over end shaking machine for 5 hours, let stand over night, then shake again for 3 to 4 hours. Shaking by hand is much less effective, and unlikely to give comparable results. After the shaking is finished, pour the contents of the bottles upon fluted filters. The filters should be previously moistened with dilute HCl and washed free of acid by water, so that they will not change the pH or PO_4 content of the extract. Some of the first of the filtrate is rejected. After the remainder is collected, the pH is determined and the PO_4 is estimated by the molybedenum blue method. Parker's (19) procedure is followed.

In this way, three values of pH and PO_4 for each soil are found. These figures may be plotted; then from the graph, the PO_4 of the soil at exactly pH 4 may be read off.

This test gives information in regard to the buffer power of the soil, how it will be affected by acid, and how much PO_4 will be dissolved at pH 4 or some other convenient point.

Rate of solubility curve. By using several different ratios of soil to solvent, Vanstone (25) derives from the results a curve indicating the relative availability of soil phosphate. In his examples, the chemical tests seem to agree fairly well with field results. Similar results are given by Simon (22). In applying this method to several of the soils used in this work the results do not show the same availability of soil PO_4 as is shown by pot and plot tests with plants. The laboratory results are set out in Table 6. Just as in other equilibrium studies, the figures obtained for most soils show fairly well the relative availability of the soil PO_4. But some marked exceptions with all these methods greatly reduce the value of the results as a means of estimating the fertility of an unknown soil. In this respect, the Vanstone method seems no better than most other equilibrium methods. In all the modifications of this method here tried, the results obtained for soils 38, 64, and 80 do not agree with results of pot cultures. In the latter, soil 38 has generally shown a good availability of PO_4 to tomatoes, alfalfa, and barley, whereas soils 64 and 80 fail to give satisfactory crops without added PO_4, yet the chemical equilibrium tests indicate that these soils contain more available PO_4 than soil 38.

Percolation methods.

The percolation method of extracting PO_4 from a soil is more like the action of plants than the equilibrium method, since it removes from the soil the products of the action of the solvent, and thus permits the reaction to go toward completion in one direction. In beginning this study, it was hoped that percolation methods would give some measure of the power of a soil to renew PO_4 in the soil solution after the easily soluble PO_4 had been removed. J. W. Tidmore and L. Meyer, working independently in this laboratory, at about the same time sought to attain the same end by different methods (see p. 199).

The methods, described in the following, have been in use here for three years, have been applied to hundreds of different soils, and have given results in most cases according fairly well with the character of the soil as shown by growth of plants in pots or in the field.

Two concentrations of acid, 0.001 *N* and 0.05 *N*, have been used. The procedure is essentially the same with both, though the apparatus used with the more dilute acid is capable of much more precise control so that the results are more easily reproducible.

The details of procedure are about as follows, so far as the soil is concerned.

Twenty grams of the soil is placed in a filter tube 48 mm. in diameter. The soil rests on a bed of filter paper pulp 5 to 8 mm. thick, supported on a filter plate. If the soil is fine in texture, some acid-washed white sand is mixed with it to aid in percolation. This sand is quite fine, nearly pure quartz. It has a slight fixing power so that a small amount of PO_4 is retained by the sand when a dilute solution is passed through it. However, it was found that after 1 litre of 0.001 *N* HCl had been passed through it, practically the whole 0.2 mgm. of PO_4 added to the sand was recovered. So there is no fear that the sand will retain any of the PO_4 extracted from the soil.

When the filter tube with the soil and with added sand, if necessary, is set up it is connected with an elevated reservoir of the dilute acid by a pressure tube about 1·6 m. long, and the acid is allowed to percolate through the soil at the rate of about 1/3 liter an hour continuously for about 20 hours until very little PO_4 is found in the percolate or until a definite volume of percolate has been collected. Originally the percolation was continued until nearly complete extraction of the PO_4 soluble in that reagent was obtained. Lately the procedure has been standardized so that 7 litres of percolate are taken for each soil.

Percolation with 0.001 N *HCl.* With this procedure, the percolate is collected in a train of litre bottles in such manner that the contents of the seven bottles represent successive stages in the extraction. The percolates are analyzed separately so that the rate of extraction of PO_4, and the Ca and the pH of each

may be determined. When the apparatus is set up and percolation started, it proceeds automatically without attention except to see that the supply reser-

TABLE 9.

PO_4 extracted from soils by percolation with 0.001 N and 0.05 N HCl.

Soil number	Acid normality	PO_4 in successive 1-litre portions of percolate							Total extracted	Total in soil	Available
		1	2	3	4	5	6	7			
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*mgm.*	*p.p.m.*	*per cent.*
1C	0.001	1.50	2.65	2.60	2.65	2.50	2.00	1.65	15.75	787	82
	0.05	17.00	1.10	0.5	0.30	0.30	0.20	0.10	19.30	965	
75	0.001	3.60	4.40	4.40	3.20	1.60	0.80	0.52	18.92	946	78
	0.05	18.00	2.70	1.00	0.54	0.54	0.54	0.40	23.76	1,188	
30	0.001	5.00	4.40	2.70	2.00	1.10	1.00	0.90	17.10	855	76
	0.05	20.00	1.00	0.50	0.30	0.30	0.10	0.10	22.30	1,115	
53	0.001	2.10	1.80	1.50	1.20	0.80	0.60	0.5	8.35	417	91
	0.05	6.40	1.60	0.50	0.22	0.20	0.14	0.10	9.16	458	
68	0.001	5.00	6.20	4.40	2.80	2.00	1.40	1.0	22.80	1,140	92
	0.05	22.00	2.00	0.40	0.20	0.10	0.10	0.0	24.80	1,240	
78	0.001	0.14	0.16	0.12	0.08	0.06	0.04	0.04	0.64	32	71
	0.05	0.40	0.16	0.10	0.08	0.07	0.06	0.04	0.91	45	
36	0.001	0.75	1.65	2.00	1.65	1.65	1.40	1.25	10.35	517	77
	0.05	11.00	0.88	0.44	0.34	0.29	0.25	0.20	13.40	670	
37	0.001	1.10	1.15	1.10	1.10	1.10	1.10	1.10	7.75	387	59
	0.05	10.80	1.60	0.31	0.20	0.16	0.12	0.08	13.30	663	
38	0.001	0.40	0.80	1.24	1.44	1.16	0.50	0.40	5.94	297	87
	0.05	4.00	1.00	0.48	0.31	0.25	0.20	0.16	6.40	320	
64	0.001	0.68	1.60	1.60	1.44	0.88	0.88	0.88	7.96	398	56
	0.05	8.00	3.20	0.80	0.70	0.60	0.40	0.40	14.10	705	
80	0.001	0.88	1.76	2.00	2.00	1.60	1.10	1.00	11.00	550	60
	0.05	16.00	2.90	0.20	0.10	0.10	0.06	0.04	19.40	970	

TABLE 9—*contd.*

PO_4 extracted from soils by percolation with 0.001 N *and 0.05* N *HCl*—contd.

Soil number	Acid normality	PO_4 in successive 1-litre portions of percolate							Total extracted	Total in soil	Available.
		1	2	3	4	5	6	7			
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*mgm.*	*p.p.m.*	*per cent.*
35	0.001	0.02	0.04	0.04	0.04	0.04	0.06	0.07	0.31	15	13
	0.05	0.66	0.44	0.29	0.25	0.25	0.22	0.20	2.31	115	
59	0.001	0	0	0	0	0	0	0	0	0	0
	0.05	0.10	0.04	0.01	0.01	0.01	0	0	0.17	8	
89B	0.001	1.16	2.00	1.36	0.80	0.60	0.54	0.40	7.06	353	44
	0.05	7.20	2.20	1.40	1.30	1.30	2.00	0.90	16.20	810	

voir of dilute acid is kept filled. When one soil is sufficiently extracted, it may be quickly replaced by another, a new set of collecting bottles is set in position and the process continues without further attention.

It is obvious that in order to have truly comparable results with different soils, all variables except the soil, should be kept out of the procedure. This is fairly well done by the apparatus used. It has been more fully described in another paper (13). The rate of flow of the dilute acid through the soil varies somewhat from moment to moment, but averages very nearly 1 litre every three hours. Starting with 20 gm. of soil, nearly all the PO_4 soluble in 0.001 *N* acid is extracted from good soils by 7 litres of percolate, but not all is removed from some of the soils having the PO_4 in

TABLE 10.

Analyses of soil percolates made with 0.001 N *HCl.*

Percolate number	pH	Ca	PO_4	pH	Ca	PO_4	pH	Ca	PO_4	pH	Ca	PO_4
		p.p.m.	*p.p.m.*		*p.p.m.*	*p.p.m.*		*p.p.m.*	*p.p.m.*		*p.p.m.*	*p.p.m.*
	Soil 38			*Soil 64*			*Soil 35*			*Soil 59*		
1	4.0	14	0.16	4.0	14	0.08	3.6	12	0	4.0	20	0
2	3.8	14	1.08	4.0	14	1.32	3.4	16	0	3.6	6	0
3	3.6	22	1.44	4.2	14	2.00	3.3	10	0	3.2	3	0
4	3.4	18	1.44	3.8	14	1.60	3.2	6	0	3.2	0	0
5	3.4	10	1·08	3.4	10	1.32	3.2	2	0.04	3.0	0	0
6	3.2	6	0.56	3·2	6	1.00	3.0	1	0.07	3.0	0	0
7	3.2	6	0.48	3.0	2	0.80	3.0	0	0.07	3.0	0	0

TABLE 10—*contd.*

Analyses of soil percolates made with 0.001 N *HCl*—contd.

Percolate number	pH	Ca	PO_4	pH	Ca	PO_4	pH	Ca	PO_4	pH	Ca	PO_4
		p.p.m.	*p.p.m.*		*p.p.m.*	*p.p.m.*		*p.p.m.*	*p.p.m.*		*p.p.m.*	*p.p.m.*
	Soil 1C			*Soil 30*			*Soil 37*			*Soil 80*		
1	6.2	4	1.50	4.6	12	5.00	6.8	5	1.10	5.2	6	0.88
2	5.6	5	2.65	3.3	1	4.40	6.2	7	1.15	5.2	6	1.76
3	4.4	7	2.80	3.2	0	2.70	5.4	12	1.10	4.2	14	2.00
4	4.2	7	2.65	3.2	0	2.00	5.4	15	1.10	3.8	10	2.00
5	4.0	7	2.50	3.0	0	1.10	5.4	12	1.10	3.4	6	1.60
6	3.7	4	2.00	3.0	0	0.95	5.4	10	1.10	3.0	3	1.10
7	3.4	3	1.65	3.0	0	0.90	5.2	8	1.10	3.0	tr.	1.00
	114 mgm. $CaHPO_4$, $2H_2O$			*66 mgm. $Ca_3(PO_4)_2$*			*0·2 gm. white rock phosphate*			*0·2 gm. black rock phosphate*		
1	3.6	..	66.00	3.4	..	40.00	3	6	15.6	3.4	15	25.0
2	3.0	..	6.20	3.0	..	0.36	3	4	10.8	3.0	12	21.0
3	3.0	..	0.96	3.0	..	0.30	3	3	7.3	3.0	8	15.4
4	3.0	..	0.34	3.0	..	0.24	3	..	6.6	3.0	..	13.5
5	3.0	..	0.26	3.0	..	0.20	3	..	6.3	3.0	..	7.3
6	3.0	..	0.24	3.0	..	0.18	3	..	5.6	3.0	..	5.2
7	3.0	..	0.22	3.0	..	0.20	3	..	5.3	3.0	..	3.1

less available condition. The rate of flow being nearly constant, and the aliquots of percolate being of the same volume, the results for different soils are thought to be properly comparable. The figures obtained for a number of soils are set out in Table 9, which also gives the figures found when 0.05 *N* acid was used. The figures reveal the great difference between soils as to solubility of PO_4. Some, such as 1C, 36, 38, 65, have considerable buffer power so that much of the free hydrogen ion of the first litre of acid passing through the soil is neutralized with the result that there is little change in pH of the soil and consequently little PO_4 dissolved. As the

buffer effect of the soil is overcome, pH is lowered and PO_4 in the percolate increases to a maximum, which depends on the rate of flow and the character of the soil.

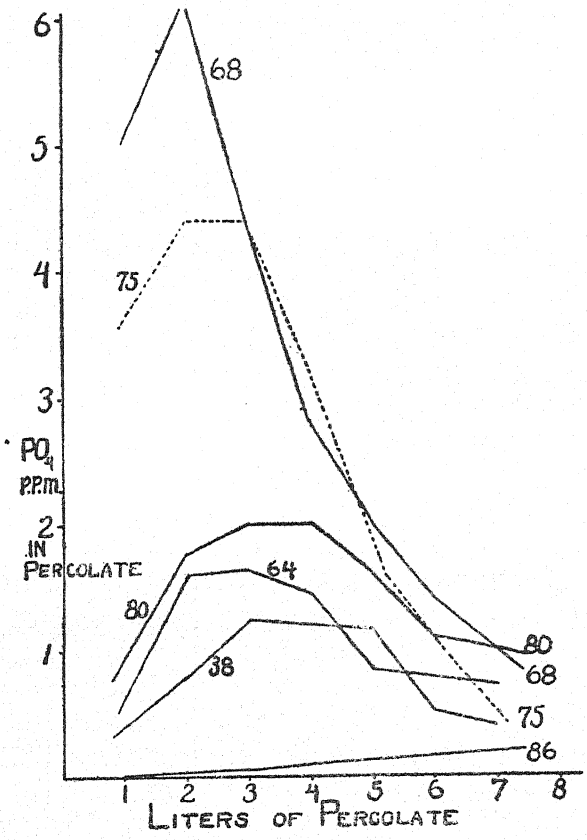


Fig. 2. PO_4 in Extracts made by continuous percolation with 0.001 *N* HCl

Other soils, such as 30, 53, 68, have little buffer power and this is soon overcome by the dilute acid so that the highest concentration of PO_4 is found in the first litre of percolate, and rapidly decreases thereafter. At first, it was thought that this indicated how the soils would respond in supplying PO_4 to a growing crop. Experience has not yet shown whether or not this is true.

In the use of this method, it is a very simple matter to determine Ca by turbidity and pH by color comparison in the separate bottles of percolate. From 1 to 2 hours' time is enough to make all the tests for two soils, in the 14 bottles. This information often helps much to understand the nature of the soil. Table 10 presents some of the figures thus found. Most of the easily soluble or replaceable

Ca, presumably Mg also, is soon removed along with the easily soluble PO_4. When the soil contains much $CaCO_3$, this neutralizes the acid and prevents lowering of pH, so PO_4 is not so rapidly extracted. This is shown by soil 37 which contains 1 per cent. $CaCO_3$. Some soils such as 35 and 59 contain a fair amount of PO_4, but it is practically insoluble in 0·001 *N* acid. In such soils, usually the amount found in the percolate increases slowly for some time as the volume of percolate increases. In these, it seems probable that the PO_4 is combined with Fe or Al in such manner that it is not soluble in 0·001 *N* HCl. When the PO_4 is combined with Ca, it is easily dissolved by this acid (Table 10, in which the action on $CaHPO_4$ and $Ca_3(PO_4)_2$ and on two rock phosphates is shown). In this work, HCl is the acid used because of its convenience and the easy solubility of its reaction products. HNO_3 works in the same way and is more to be expected in the soil solution, but it is likely to interfere in the colorimetric estimate of PO_4. HCl of 0·001 *N* strength has a pH of about 3, somewhat similar to that of water saturated with CO_2 and perhaps not greatly different from the pH of the sap of some plants. It has very little action on soil silicates or zeolites except to replace imperfectly Ca, Mg, Na and K.

Percolation with 0·05 N *HCl.* This concentration of acid was selected because it seems strong enough to remove from the soil all the PO_4 that plants are likely to be able to extract within many years. It almost completely replaces bases, Ca, Mg, Na and K. It completely extracts PO_4 from $Ca_3(PO_4)_2$ and from ordinary rock phosphates. It dissolves some iron and silica, usually very little from ordinary soils. In order to carry the extraction of PO_4 to completion with 0·05 *N* acid, the dissolved substances must be removed so that the reaction can go to completion in one direction. This is accomplished by continuous percolation.

The concentration of hydrogen ion in this acid, about pH 1·35, is sufficient to overcome quickly the buffer power of 20 gm. of ordinary soil so that the larger portion of the available PO_4 is removed by the first litre of percolate. Succeeding litres of percolate contain less and less PO_4, as shown in Table 9. On account of the rapid removal of the PO_4 by this acid, little useful information is obtained by testing each litre of percolate separately, so the whole is collected together. Some relatively infertile soils still give up appreciable amounts of PO_4 to 0·05 *N* HCl after 7 litres have percolated through 20 gm. of soil at the rate of 200 to 300 cc. an hour. This is true of soils 35 and 64, and of 59 to which considerable amounts of superphosphates have been added. Very little PO_4 is extracted from untreated soil 59. In many cases the amount of PO_4 extracted by percolation with 0·05 *N* HCl fairly well represents the capacity of the soil to supply PO_4 to crops, but perhaps not as well as the 0·001 *N* percolate.

Since carbonic acid is perhaps the most important in its effects of any free acid usually found in the soil, it would seem reasonable to use this agent for ex-

tracting PO_4 from the soil. For this purpose, a modification of the apparatus used for percolating with 0·05 *N* HCl was made in order to use water saturated with CO_2 under a head of about 1·5 m. of water. The gas was caused to bubble into the lower end of the column of water which was flowing to the percolator. From the top of this column, the CO_2 passed into the reservoir which supplied the water so that the water was kept saturated with the gas at all times, at that temperature and pressure. The pH of this solution as it passed to the soil in the closed percolator was about 3, like that of 0·001 *N* HCl, but the total acid-

TABLE 11.

PO_4 extracted from soils by various solvents, and by different procedures.

Soil number	Total by fusion	PO_4 in soil				Equilibrium with water, 1 : 1	PO_4 in solution at 1 : 5 equilibrium with acids						
		By hot HCl, 10 per cent.	By percolation with				Normality of HCl				Normality of citric acid		
			0·05 *N* HCl	0·05 *N* CO_2	Water, pH 5		0·01	0·02	0·20	1·0	0·025	0·05	0·20
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1C	2,000	1,160	680	660	307	0·18	4	10	50	200	11·4	27	66
30	1,600	1,370	1,197	702	290	8·00	40	80	166	310	35	40	88
35	2,400	1,120	140	0	0	0·12	0·16	0·24	10	44	5·6	8·8	11
36	2,500	1,020	418	420	..	0·30	2·3	9·4	36	160	13	20	36
37	2,400	..	494	829	..	0·47	0	0·2	25	160	5	..	44
38	2,500	990	320	210	40	0·22	0·8	1·2	13	57	10	13	24
40	3,300	2,720	1,430	1,100	..	1·40	13	20	166	360	33	..	160
45	3,800	..	1,850	..	..	0·40	5	13	..	..	13	..	..
46	1,600	..	80	..	..	0	1·2	2·2	..	..	5·6	..	..
59A	2,000	100	tr.	..	..	0	0	0	0·3	1	0·6	0·7	2
64	1,700	1,180	650	360	63	0·16	0·4	2	50	100	10	13	27
65	2,800	..	1,170	1,170	..	3·4	35	60	200	280	80	..	182
69A	1,600	1,210	12	0	..	0·14	0·3	0·4	1·4	100	2·3	..	3·2
78A	450	340	50	13	..	0·20	1·4	1·8	8	22	..	..	6·6
80	1,150	950	795	738	128	0·30	2·3	27	118	200	..	..	..

ity, found by titration, was about 0·05 *N* like that of the HCl used in the same way. In other words, little of the CO_2 was ionized, the solution was highly buffered, and

its solvent power approached that of 0·05 *N* HCl. In Table 11, column 5, are given a few figures showing the results obtained by the use of this acid. Since it is very inconvenient to use, and the results it gives are no more significant than those obtained with HCl, its use was not long continued. Although the water solution of CO_2 entering the percolator had a pH of about 3, the solutions coming from the percolator, when the pressure no longer confined the gas, had a pH near 5.

More dilute solutions of CO_2 in water were tried but their solvent power was so little that they seemed impractical. Water containing CO_2 at pH 5, which is about that of ordinary distilled water, must be percolated through a mass of 20 gm. of good soil for many days in order to extract as much PO_4 as will be removed by 7 litres of 0.001 *N* HCl in 15 hours (Table 12). For the practical purpose of estimating the easily available PO_4 in a soil, water containing none or only small amounts of CO_2 is very ineffective. A greater concentration of hydrogen ion than can be had in this way is necessary to produce much solvent effect.

In Table 11 are presented the results of analyses of several different soils by a number of different procedures with various solvents of different strengths. There are three subdivisions of the table; first, the total PO_4 found by fusion, and by hot 10 per cent. HCl; second, PO_4 found by percolation; and third, the PO_4 found in equilibrium extracts. The figures are thus assembled to facilitate comparisons of the different methods. In most cases, the total PO_4 found by fusion and that extracted by strong hot acids, has little relation to the value of the soil as shown by plants, so that such determinations are generally useless for indicating the fertility of the soil. Equilibrium extracts with dilute weak acids supply much more useful figures. The best indication is the result found by percolation with very dilute acids. This will be considered in more detail later.

TABLE 12.

*PO_4 extracted by percolating with ordinary distilled water**

Soil number	PO_4 in soil
	p.p.m.
1C	307
30	290
35	0
38	40
64	63
80	128

* The same apparatus and procedure were used as with 0.05 *N* HCl.
Volume of percolate, 6-7 litres. In all except 35, there was still much PO_4 in the last percolate, showing that the soil was not nearly exhausted of water-soluble PO_4 by this method.

Relative solubilities with different solvents. The amount of PO_4 extracted from soil by any of the aforementioned methods, either by equilibrium or by percolation, does not always indicate whether or not the PO_4 thus obtained will be supplied to plants by the soil in such manner or rate that it will support good growth of crops. Other students of this subject have reached a similar conclusion. To provide a more reliable or adequate notion of availability, the factor "relative solubility" has been introduced. One of its most prominent advocates is Lemmermann (14), who has contributed numerous papers on this subject. He extracts soil with hot 10 per cent. HCl and with 1 per cent. citric acid. When the PO_4 extracted by the latter is less than 25 per cent. of that dissolved by the HCl he considers that the soil is likely to respond to phosphate fertilizers. In this laboratory, a number of soils treated by this method gave results not in accord with their behavior in pot cultures. It may be that the amount and concentration of the acids used by Lemmermann are not well adapted to our soils. The principle seemed worthy of further study. It was applied to a number of combinations of different solvents without giving results of much value, except in one case. The results are given in Table 13.

The Lemmermann numbers shown in this table in the column headed 100 C/B show some of the soils in their proper relations, but others are far from correct; e.g. 59, 64, 78, the poor soils, are made to appear better than they really are. In the

TABLE 13.

Relative solubilities of soil phosphate.

Soil number	RELATIVE SOLUBILITY		
	100 C / B*	100 D / A	100 E / D
1C	5.7	48.0	82
30	6.4	70.0	76
35	1—	6·0	7
36	3.5	27.0	77
38	2.4	13.0	87
40	5.8	43.0	60
53	5.6	57·0	91
59	2.0	0.0	0
64	2.3	39.0	56
69	0.2	0.7	0
78	2.0	10.0	71
80	..	84.0	60

* 100 *C/B* is Lemmermann's factor of availability.

A=p.p.m. PO_4 in soils found by fusion (see Table 11).

B=p.p.m. PO_4 in soils found by equilibrium hot 10 per cent. HCl (see Table 11).

C=p.p.m. PO_4 in soils found by equilibrium 0.2 *N* citric acid (see Table 11).
D=p.p.m. PO_4 in soils found by percolation with 0.05 *N* HCl (see Table 9).
E=p.p.m. PO_4 in soils found by percolation with 0.001 *N* HCl (see Table 9).

column headed 100 D/A the valuations are more nearly right, although in this, soils 38 and 64 are not in proper relation to each other. In the column headed 100 E/D 38 and 64, as well as all the others, receive correct values.

Relative solubility of soil PO_4 in 0·001 N *and in 0·05* N *HCl, by percolation.* In all cases so far observed, it is found that if the PO_4 extracted by percolation with 0.001 *N* HCl is less than half as much as is found by similar treatment with 0·05 *N* HCl, the soil is likely to be deficient in power to supply phosphate to plants. On the other hand, good soils give up nearly as much PO_4 to the weaker as to the stronger acid. The results for a number of soils are given in Table 9 in the last column of which is shown the percentage of PO_4 by percolation with 0·001 *N* HCl of the amount found by 0.05 *N* HCl. This gives soils 64 and 80 a low ratio of availability of PO_4 although the amounts extracted are higher than for soil 38, which gives good results in pot cultures without added PO_4, whereas 64 and 80 require phosphate in order to produce good crops. When the absolute amount of PO_4 extracted by either of these acids is very low, as in soil 78 A, crops respond to added phosphate even though the relative solubility of that originally present is high.

TABLE 14.

Phosphate-supplying power of soils.

As related to particle size—per cent. clay × p. p. m. PO_4 extracted with 0.05 *N* HCl by percolation.

Soil number	Clay	PO_4	Clay × PO_4
	per cent.	*p.p.m.*	
10	42	680	28,560
21	5	525	2,625
30	7	1,197	8,400
35	29	140	3,060
36	30	418	12,540
37	39	494	20,000
38	52	320	16,640
40	31	1,430	44,330
44A	40	100	4,000
53A	4	500	2,000
54A	42	175	7,350
55A	37	860	31,820
56A	40	490	19,600
59A	48	tr.	48
64	10	350	3,500
65	32	1,170	37,440
66A	42	6	228
68A	12	1,000	13,200
69A	50	12	600

Amount of phosphate in various sized particles.

An attempt to explain the relative fertility of soils 38 and 64 on the basis of the amount of PO_4 in large and small sized particles gave no useful information. The soils were separated into sand, silt, and clay fractions by sedimentation in water. The PO_4 in the various fractions was determined but the figures obtained were so similar for the two soils that the differences seemed inadequate to explain the great difference between the two soils respecting available phosphate as indicated by the growth of plants.

It is evident that the physical texture of a soil may greatly affect the availability of the soil phosphate. A fine textured soil has vastly more points of contact for plant roots than a coarse soil, so that a much lower concentration of PO_4 in the fine soil may furnish a more adequate supply to plants than would a coarse soil having a much higher content of PO_4 in its larger particles.

Failyer, Smith and Wade (10) indicate that in most of the soils examined, the concentration of PO_4 in various sized particles is greater as the size of the particles is smaller.

An attempt was made to correlate the availability of soil phosphate with the proportion of fine material in the soil. The percentage of clay in the soil was multiplied by the number of parts per million of PO_4 found by percolation with 0.65 *N* HCl. In general, the higher the figure thus obtained, the greater is the amount of available PO_4 in the soil. But the availability of PO_4 in some sandy soils such as 30 and 68, is much underestimated by this method, whereas soils 38 and 64 receive a relatively proper value. The figures obtained by this calculation are shown in Table 14.

TABLE 15.

Comparison of phosphate extracted by equilibrium and by percolation

Soil number	PO_4 in soil					
	Equilibrium extracts, 1 : 5 with			1 : 10 with 0.2 *N* HNO_3 Fraps method	Percolation extracts, with HCl	
	0.02 *N* HCl	0.20 *N* citric	1.0 *N* HCl		0.001 *N*	0.05 *N*
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1C	50	66	200	400	787	965
30	166	88	310	1,120	855	1,115
35	10	11	44	80	15	115
36	36	36	160	220	517	670
37	25	44	160	250	387	663
53	100	44	160	500	417	458
59	0.3	2	1	2	0	8
38	13	24	57	80	297	320
64	50	27	100	270	398	705

Relative amounts of phosphate extracted from soils in equilibrium extracts compared with amounts found by percolation with different acids.

The data shown in Tables 11 and 12 permit a comparison better shown by recombination of the figures in Table 15.

It is apparent that the amount of PO_4 extracted increases as the strength of the acid is increased and that very much more PO_4 is obtained by percolation with the dilute acids than in equilibrium extracts made with much more concentrated acids. Exceptions are soils 35 and 59 A which have relatively very little available phosphate. Such soils would show no available PO_4 to the dilute acid used in percolation if it were not for the large volume of acid used in percolation.

It appears to be a general law with respect to solubility of soil phosphate that PO_4 dissolved increases as concentration of hydrogen ion increases, and also as the volume of solvent is increased.

From Table 11, it may be seen that there is no relation between the total PO_4 in a soil and the available PO_4 extracted by dilute acids.

Effect of several salts on solubility of soil phosphates.

To 100 cc. of water were added 50 gm. of soil and 1 gm. of the salt. After being shaken 1 hour, the mixture was filtered and the filtrate tested for PO_4. Soils 1C, 36, 37, 38 were used in the experiments. When $FeSO_4$, $CuSO_4$, and $CuCl_2$ were used, no PO_4 was found in the filtrates.

With NH_4Cl, $(NH_4)_2SO_4$, NH_4NO_3, $CaCl_2$, $CaSO_4$, $BaCl_2$, NaCl, Na_2SO_4, KCl, and K_2SO_4 there was about as much PO_4 in the extracts as with distilled water alone. Ammonium oxalate and ammonium citrate gave extracts containing about 10 times as much PO_4 as did distilled water.

There seemed to be no useful information obtained in this way, so the study was not long continued.

Methods proposed by others.

In the course of this study, several methods proposed by others have been studied briefly. The work of von Wrangell and collaborators (27, 28, 29, 30, 31) is most instructive. Time and suitable equipment have not been available to the writer to repeat or verify the very interesting, painstaking, and fundamental work of these investigators in respect to soil phosphates and their availability to plants.

Von Wrangell's work.

Shortly after the main group of papers from these workers were published, there appeared in *Chemical Abstracts* brief mention of them, but these abstracts did not make evident the importance of the work done. Indeed, it seems probable that

few except German workers in this field have yet heard of, or become impressed by, this work. The writer did not know of it until after the work here reported was done. The conclusions of von Wrangell seem to be supported by such work done in our laboratories as has any bearing on the subject.

The chief factors to be considered in evaluating the ability of a soil to supply PO_4 to growing plants have been very well enumerated by von Wrangell (27, p. 637): (*a*) The kind of plant, (*b*) presence of lime salts, (*c*) effects of electrolytes and organic substances in the soil, (*d*) the reaction and absorbtive power of the medium in which the plant grows, (*e*) action of microflora. Also it is important to know the time rate at which a soil will regain its original concentration of PO_4, after it has been depleted by extraction with water or by growing plants.

In executing the von Wrangell method, 1 gm. of soil is shaken five hours with 100 cc. of water. The solution is clarified by centrifuging, the clear fluid is siphoned off, and the soil is again shaken with 100 cc. of water as at first, and thus a second extract is obtained. Sometimes further extractions are made in the same manner.

The PO_4 found in the two extracts is designated a and b, then by the formula $a^2/a\text{-}b = x$, is found the total available PO_4 in the soil. If the necessary machines are available the method is short, simple, and convenient. Prof. von Wrangell states that the results obtained are in good accord with the results of pot cultures and field tests.

The writer attempted to obtain similar results by separating the soil extract by filtering, as a suitable centrifuge was not available. This makes the method tedious and less exact than that proposed by von Wrangell, but the results seemed to agree, for most of the soils tried, with the known character of the soils in respect to capacity of the soils to supply phosphate to plants. If this method is found by general experience to prove as reliable as its author predicts, it will, perhaps, replace most other methods now in use.

For estimating the availability of phosphate in calcareous soils, Das (6) has suggested the use of potassium carbonate. Such a reagent may hydrolyze iron and aluminium phosphates, making PO_4 soluble, also replacing Ca in $CaHPO_4$, thus bringing PO_4 into solution. Such an alkaline reagent also dissolves much organic matter, including some organic phosphorus. It is difficult to separate organic from inorganic phosphorus, also the solution, is troublesome to prepare for analysis by the molybdenum blue method. Some trials of the methods in this laboratory indicated that the results obtained by it have little relation to availability of the phosphate in nearly neutral soils.

The Neubauer (15) method for determining the availability of soil phosphate has not been investigated during this study. It is a plant culture, not a chemical method. A good comparison of the Neubauer and Lemmermann methods is given

by Engels (9) and by Blanck (3). In this country, Neubauer's method for potassium has been studied by Ames and Gerdel (1), and by Haley and Holben (12). As applied to phosphate, there seems to be no published report of work with the method in this country.

Arrhenius (2) proposes to extract the soil with a 1 per cent. solution of NaCl containing enough H_2SO_4 to make the solution about 0.002 *N* in hydrogen ion. As might be expected, the writer found that the PO_4 thus extracted is about the same as if the dilute acid alone were used. This method does not give proper relative values to the power of the soils examined to supply plants with available PO_4.

Dirks and Scheffer (7) add to 100 gm. of soil, 1 gm. $CaCO_3$ and 250 cc. water, then pass CO_2 through the mixture. After some hours, PO_4 is determined in the filtrate. The results obtained in this laboratory by this method were not in agreement with the results shown by plants on the same soils.

Christensen (5) has proposed the use of Azotobacter as an indicator for availability of soil phosphate. His method has been modified and used by Niklas and others (16, 17, 18). A trial of it in this laboratory failed to give useful results. Yet Winogradsky (26) says it gives reliable results in 85 per cent. of the tests.

Dyer (8), in 1894, proposed 1 per cent. citric acid as an agent for determining available soil PO_4. Much useful information has been obtained in this way, yet the results are not always satisfactory.

Many investigators [notable among them is Fraps (11)] have used 0·2 *N* HNO_3 for determining the availability of soil phosphate. Some have used much stronger acids, *e.g.* Lemmermann who used hot 10 per cent. HCl compared with 1 per cent. citric acid to give his factor for " relative solubility " as already explained (p. 193). To the writer, it seems that the use of so drastic a reagent as hot 10 per cent. HCl to extract available PO_4 from soil is unlikely to indicate the behaviour of the soil with growing plants.

The opposite extreme is found with those who use water only to determine PO_4 in soil. Various ratios of soil to water have been tried, all the way from the displaced soil solution as obtained by Burd and Martin (4), to the 1 to 100 ratio used by von Wrangell. Undoubtedly the soil solution indicates what the plant has to deal with at the time the extract is made, but since the soil solution may change from day to day, it does not show what the plant can obtain from the soil over a considerable period of time. This might be expected, since only a small amount of soil phosphate which is available to plants from soils having a good supply of available phosphate, is dissolved in the soil solution at any one time. The same is probably true in regard to soil extracts made with larger ratios of water to soil unless it be that when the ratio is wide, as in the method of von Wrangell (1 soil to 100 water), all the easily available PO_4 is brought into solution.

Meyer, a collaborator with von Wrangell (31), obtained somewhat useful results in this laboratory in 1927 by making repeated extractions of the same small portions of soil with 100 times as much water, a variation of the von Wrangell method. The plotted results gave a curve indicating fairly well the availability of the soil phosphate.

At about the same time, J. W. Tidmore in this laboratory made successive diffusions of a small amount of soil in collodion bags suspended in water, by the method of Pierre and Parker (20). The amount of PO_4 found in the successive diffusates was plotted to produce a curve which indicated fairly well the ability of the soil to supply phosphate to plants. The process was prolonged and tedious though not requiring a great deal of time or attention from the operator. Much time was required because a considerable fraction of the PO_4 dissolved by the water remained in the collodion sack with the soil each time when the diffusate was removed from the outside container. Although the figures obtained by Meyer and by Tidmore in most cases gave a useful indication of the power of the soil to supply phosphate to plants, the results were not always in accord with the growth of plants in those soils as related to available phosphate.

Apparently no one of the published methods has been found always reliable, so the search still continues.

Conclusions.

In the opinion of the writer, a satisfactory estimation of the availability of soil phosphate by extraction with any one solvent of whatever strength, will not, in some cases, be possible. Relative solubility and the total PO_4 dissolved in two solvents of quite different strength, but neither of them strong enough to cause much decomposition of the soil, provide figures much better indicating the capacity of the soil to supply plants with phosphate.

A fairly reliable estimate of the phosphate-supplying power of an unknown soil may be most easily and quickly obtained by analysis of the extract made by the acid of a dilute acid which has been added in such amount that the extract will have approximately pH 4. When there is some uncertainty in regard to the availability of the phosphate thus found, the more certain, as well as more laborious, method of "relative solubility" should be applied.

The equilibrium test will at once indicate the very poor and the very good soils, with the least time and expense. The percolation tests will give more reliable information in uncertain cases.

The only certain means of determining the response of a soil to fertilizers lies in the actual trial with plants in greenhouse or field.

SUMMARY.

Some reasons why a satisfactory estimation of the availability of soil phosphates by chemical methods is difficult are :

Phosphate found in soils is mostly present in relatively slightly soluble combinations, so that the concentration in the soil solution is never high.

Plants have selective action in absorption of nutrients, and also it seems probable that plants are able to take up ions such as PO_4, K, perhaps others from films of solution not represented by solutions prepared in the laboratory.

No chemical agent can imitate these effects of plants on soils.

Many soils have such high fixing power that an easily soluble phosphate added to them is quickly fixed, thus becoming more or less unavailable to plants.

Deficiency of other necessary plant nutrient in the presence of sufficient phosphate, or presence of excessive amounts of soluble salts or toxic substances in the soil, may cause the failure of plants.

Test of a soil some time after phosphate has been added and after plants have grown on it subsequent to addition of the phosphate cannot show the condition of the soil with which the plant had to deal at the start.

Equilibrium, 1:5 extracts of soils with dilute acids provide the simplest and quickest means of obtaining some idea of the available phosphate, although such extracts do not always give a correct idea of the relative power of different soils to supply phosphate to plants. Extracts made with three or more different concentrations of an acid give a much better picture of the available PO_4 than is obtained by any single extract. Extracts thus made show only the PO_4 available at the time of extraction, not the relative supplying power of the soil for any great length of time.

Citric and oxalic acids, because they form soluble complexes with Fe, Al, Ca, and Mg (the cations which tend to repress solubility of PO_4), are not appropriate for determining the availability of soil phosphates.

A solution of CO_2 in water would be very appropriate for the purpose if it were easily possible to duplicate conditions at will, but its application is too difficult.

Dilute K_2CO_3 hydrolyzes some phosphates and thus brings PO_4 into solution, but the results do not seem to have much bearing on availability.

If results of tests of different soils are to be compared, the buffer power of the soil must be reckoned with. The amount of acid used in making an extract should be varied according to the buffer power of the soil so that the extracts of all soils will have the same pH, e.g. 4. This means that some soils will need two or three times as much acid as others to have an extract with pH 4.

Since it is difficult to prepare a soil extract of exactly pH 4, or other definite figure, the PO_4 dissolved at any such definite point may be found exactly, by making three extracts with three different concentrations of acid, plotting the results and from the graph finding the PO_4 at the desired pH. For this purpose, a highly buffered acid is desirable. Acetic is very appropriate, much better than HCl.

The amount of PO_4 dissolved increases as the volume of solvent is increased, up to a large dilution. Water extracts of some soils have almost the same concentrations of PO_4 regardless of the volume of water used, up to a dilution of 100 or more water to 1 soil.

The results of such equilibrium extractions seem to be more in accord with plants' response the nearer the proportion of water to soil is to that in actual soil. But since such extracts are difficult to prepare, a ratio of 1 soil to 5 water serves very well and is more convenient.

Vanstone's "rate of solubility curve" does not in all cases express the plant availability of soil phosphate. It seems to offer little advantage over other equilibrium methods.

Percolation methods of making soil extracts resemble the action of a plant more than equilibrium methods, yet do not show the power of the soil to continue supplying PO_4 so well as was expected and hoped. In these methods the soil is percolated slowly with very dilute acid till most of its soluble PO_4 is removed. An automatic apparatus for percolation has given very good results. Curves formed by plotting the results from percolation extracts are characteristic of the soil's individual power to supply phosphate.

Percolation with 0.05 *N* HCl is supposed to dissolve from a soil all the PO_4 that may be expected to be available to plants for many years.

Carbonic acid, 0.05 *N*, has much the same solvent power as HCl, but is very inconvenient.

Water alone, or containing only a little CO_2, is so poor a solvent for soil PO_4 that it seems inadequate as an agent for estimating availability by percolation methods.

Relative solubilities. No single method of extracting PO_4 from soil, whether by equilibrium or by percolation, has been found always adequate to show whether a soil will supply phosphate to plants in sufficient amount and at a rapid enough rate to support satisfactory plant growth. But the *relative solubility* in two reagents of different power seems to give a figure which indicates very well the phosphate value for many soils. When the PO_4 found by percolation with 0.001 *N* HCl is 60 per cent. or more of the amount found by percolation with 0.05 *N* HCl, the soil will supply plant growth adequately, provided the total found by 0.001 *N* HCl is 300 p.p.m. or more. When the PO_4 extracted by the more dilute acid is less than 50 per cent. of the amount dissolved by the 0.05 *N* acid the soil is likely to be deficient in supporting plant growth even though the total amount found by the 0.05 *N* acid is adequate, provided it were available. The greater the difference in the amounts extracted by the two different concentrations of acid, the more likely is the soil to be deficient in supplying phosphate.

In general, more of the PO_4 in soils is contained in the fine than in the coarse particles, and the finer the particles, the more available the PO_4 will be to plants. But when the percentage of clay and silt in soils was multiplied by the number of parts per million of PO_4 found in the soil, the figures did not always properly represent the PO_4-supplying power of the soil.

PO_4 dissolved from soil by an acid increases as the pH of the soil decreases and as the ratio of solvent to soil increases.

In making equilibrium extracts 1 per cent. of a number of common salts added to the water did not much change the amount of PO_4 dissolved. Salts of iron and copper greatly decreased the PO_4 dissolved.

The method proposed by von Wrangell appears very promising so far as the writer has been able to test it, which is very little.

The methods proposed by Arrhenius, extraction with 1 per cent. NaCl in 0.002 N H_2SO_4; by Dirks and Scheffer, extraction with CO_2 and $CaCO_3$; by Christensen's Azotobacter method; by Dyer's 1 per cent. citric acid extract; by Fraps' 0.2 N HNO_3 method; by water extracts with varied proportions of soil to water; and by Lemmermann's "relative solubility" method; have been examined and none of them found generally successful with all kinds of unknown soils, though all of them may give quite useful information when applied to the kind of soil for which they were designed.

Successive water extracts made by the method of diffusion with collodion bags give a much better indication of the continuous supplying power of the soil than do single equilibrium extracts but do not with certainty give proper relative values to all soils.

REFERENCES.

(1) Ames, J. W., and Gerdel, R. W. 1927. Potassium content of plants as an indicator of available supply in soil. *Soil Sci.* **23**: 199—216.

(2) Arrhenius, O. 1929. The phosphoric acid question in the cultivation of sugar-cane in Java. *Chem. Abs.* **23**; 2780.

(3) Blanck, E. 1926. A contribution to the determination of the soil's need for fertilizer. *Ztschr. Pflanzenernahr. Dungung. u. Bodenk.* (B) **5**: 118; also *Jour. Landw.* (1928). **75**: 343—52.

(4) Burd, J. S. and Martin, J. C. 1923. Water displacement of soils and the soil solution. *Jour. Agr. Sci.* **13**: 265—295.

(5) Christensen, Harald 1915. Studies on the influence of soil conditions on bacterial life. *Centbl. Bakt.* **43** (II): 1—166.

(6) Das, S. 1926. Determination of PO_4 in calcareous soils *Chem. Abs.* **20**: 2038.

(7) Dirks, B., and Scheffer, F. 1928. Rapid methods for the determination of the phosphorus requirements of soils. *Landw. Jahrb.* **67**: 779.

(8) Dyer, Bernard. 1894. Analytical estimation of probably available mineral plant food in soils. *Trans. Chem. Soc.* **65**: 115—167.

(9) Engels, O. 1926. Comparison of the methods of Neubauer and Lemmermann. *Ztschr Pflanzenernahr. Dungung. u. Bodenk.* (B) **5**: 537.

(10) FAILYER, G. H. SMITH, J. G., AND WADE, H. R. 1908. The mineral composition of soil particles. U. S. Dept. Agr, Bul. Soils Bul. 54.

(11) FRAPS, G. S. 1909. Active phosphoric acid and its relation to the needs of the soil for phosphoric acid in pot experiments. Texas Agr. Exp. Sta. Bul. 126.

(12) HALEY, D. E. AND HOLBEN. F. J. 1927. A biological measurement of available soil potassium. *Soil Sci.* **24**: 345—350.

(13) HIBBARD, P. L. 1930. An apparatus for percolation at a uniform constant rate, etc. *Indus. and Engin. Chem.* Anal. Ed. 2: 404.

(14) LEMMERMANN, O. 1923, 1926. Methods for determination of relative solubility of soil phosphate. *Ztschr. Pflanzenernahr. u. Dungung.* (A) **2**: 363—70; (B) **5**: 105—113.

(15) NEUBAUER., H., AND SCHNEIDER, W. 1923. Food consumption of germinating plants and their use in the determination of the fertilizer requirements of soils. *Ztschr. Pflanzenernahr. u. Dungung.* (A) **2**: 329—362.

(16) NIKLAS, H., AND HOCK, A. 1927. The biochemical Azotobacter method for testing the phosphate needs of soil. *Ernahr. Pflanze.* **19**: 297—300.

(17) NIKLAS, H., SCHARRER, K., AND STROBEL, A. 1926. Phosphate solubility and growth of Azotobactor. *Landw Jahrb.* **63**: 387—410.

(18) NIKLAS, H., AND HIRSCHBERGER W. 1925. A new method for the rapid determination of the phosphoric acid requirements of our soils. *Chem. Abs.* **19**: 1610.

(19) PARKER, F. W., AND FUDGE, J. F. 1927. The colorimetric determination of organic and inorganic phosphorus in soil extracts and in the soil solution. *Soil Sci.* **24**: 109—117,

(20) PIERRE, W. H., AND PARKER, F. W. 1927. The use of collodion sacks in obtaining clear soil extracts for the determination of water-soluble constituents. *Soil Sci.* **23**: 13—32.

(21) RUSSELL E. J. 1921. Soil Conditions and Plant Growth, p. 120. Longmans, Green and Co., London.

(22) SIMON, R. H. 1930. Effect of phosphate and lime upon the rate curve of solubility of a Wooster silt loam soil. *Soil. Sci.* **29**: 71.

(23) TEAKLE, L. J. H. 1928. Phosphate in the soil solution as affected by reaction and cation concentration. *Soil Sci.* **25**: 143—162.

(24) TRUOG, E. 1930. The determination of readily available phosphorus in soils. *Jour. Amer. Soc. Agron.* **22**: 874.

(25) VANSTONE, E. 1925. Available phosphate in soils. *Jour. Agr. Sci.* **15**: 460.

(26) WINOGRADSKY, S. 1928. Note on the Azotobacter test for available phosphate in soils. *Compt. rend. Acad. Sci. Paris* **187**: 161—165.

(27) WRANGELL, M. VON 1926. I. Soil phosphate and need for phosphate. *Landw. Jahrb.* **63**: 627—668; II. Colorimetric method for determination of PO_4. *Ibid.* **63**: 669—675.

(28) WRANGELL, M. VON 1930. III. The determination of available plant nutrients in the soil *Ibid.* **67**: 149—169.

(29) WRANGELL, M. VON AND HAASE 1926. IV. Phosphate content of natural soil solution. *Ibid.* **63**: 707—738.

(30) WRANGELL, M. VON AND KOCH, 1926. Solubility laws as applied to tertiary phosphates. *Ibid.* **63**: 677—706.

(31) WRANGELL, M. VON, AND MAYER, L. 1926. V. A study of the root soluble portions of soil phosphoric acid, a comparison of the Neubauer method with the expressed solution colorimetric method. *Ibid.* **63**: 739—775.

Brief abstracts of articles I-V appeared in *Chem. Abs.* (1927) **21**: 2752, 2857, 4003.

AIR SURVEY IN RELATION TO SOIL SURVEY.

Imperial Bureau of Soil Science Technical Communication No. 19.

BY

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INTRODUCTION.

Problems of soil survey vary greatly according to the area to be surveyed. Reconnaissance surveys of large undeveloped areas which are to be mapped on the scale of several miles to the inch cannot be carried out by methods suitable for the intensive examination of arable land where single fields are of importance. Again, areas for which no special topographical maps exist cannot be dealt with in the same way as those which have been thoroughly mapped. In the Empire overseas it is often desired to map the soils and vegetation of areas containing few or no roads: ground work in such cases is extremely slow. Many soil problems require a preliminary survey for which the ordinary methods are far too slow and too costly. It is in such cases that the application of air survey is especially appropriate. The urgent need for greatly increased information as to the soil resources of the less developed parts of the world has given impetus to the development of aerial photography for the different kinds of survey work, and the Bureau has therefore asked Mr. Bourne, who has been closely identified with such work at home and abroad, to write the attached Technical Communication which it is hoped will stimulate interest in this important method of adding to our knowledge of soils of the Empire.

July 1931.

Historical.

The list of references attached is not exhaustive, but it is a fair indication of the general trend of events in the application of air survey to scientific problems other than those of archæology or topographic survey. Several scientists engaged in observational work during the war realised some of the possibilities of this new weapon and subsequent development in its use has been rapid. At first attention was concentrated on the solution of survey problems which presented abnormal difficulties from ground level. The experience gained in 'spotting' submarines was turned to practical account in mapping submerged sand-banks and shoals. Visual observation and photography were employed in the preliminary reconnaissance of unexplored territories, and the mapping of forests over vast and often inaccessible tracts was commenced. At the same time, in the sphere of topographical survey, much research was devoted to the perfection of methods based on a combination of vertical and oblique photography. The frequent need of employing the latter in time of war is obvious, and on economic grounds, in consideration of the large area covered by an oblique in comparison with a vertical photograph, some use of obliques in times of peace was clearly important.

(a) *Early developments.*

With regard to some of the possibilities of air survey in hydrographic surveys or geographical reconnaissance, special attention should be drawn to the early work of W. T. Lee, of the U. S. Geological Survey, entitled 'The Face of the Earth as seen from the Air' (16). The book is beautifully illustrated and contains an intelligent forecast of many of the subsequent developments in air survey. In the matter of geological survey, Lee wrote in 1922:—'It is perhaps premature to say much of the use of the airplane in the study of geology until it has been thoroughly tested. But it should be possible from the air to locate and map ore bodies, metalliferous veins, and outcrops of rock; for it is well known that rocks at the outcrop differ in colour, in the forms of erosion developed in them, and in the kinds of plants which they support. It is of interest that Colonel Alfred H. Brooks, who was Chief Geologist of the American Expeditionary Forces in France during the war, found that geologic boundaries could be recognised on air photographs and that by means of these photographs he could correct existing geologic maps and identify formations in inaccessible areas within the enemy lines. His method was to use air photographs in the study of geologic formations accessible to him. Then, having familiarised himself with the appearance of the different rock formations and structures on the photographs, he was able to

recognise the same features on photographs of areas held by the enemy and so project his mapping over into inaccessible territory.'

With regard to early applications of air surveys to soil surveys, A. M. Narraway, Chief Aerial Surveys Engineer, Department of the Interior, Canada, has recently written*:—'During the seasons of 1924, 1925 and 1926, some work of this nature was carried on in connection with our land classification surveys in Western Canada. Since that time little has been done as this class of surveys was practically abandoned. However the aerial work has disclosed that the photographs, properly interpreted, do show main soil characteristics beyond doubt as, for instance, sand areas on the one hand or silt deposits on the other. Where soil has been derived from the weatherings of local rock, informatian is given. Again, taken in conjuction with vegetation, something definite can be done. The principle upon which all our aerial survey work is based, is that the aerial photograph supplements, but does not displace, ground work. In other words, we assume that the field investigator is expert in his own particular work and that he will find in the wealth of detail of the photograph a means for relieving him of considerable travel and labour as well as for avoiding the necessity of making a map. The Photograph itself is the map and is on a scale of, say, 1,000 feet to the inch. Notes are placed directly on the photograph in the field, and the boundaries of types are usually visible on the photograph, or can be easily placed thereon by reference to trees or topographical features. Regarding the differentiation of soils within the main classes, the photographs will certainly help in a mapping way and, no doubt, will in the hands of an expert in the field assist in the identification as well '.

With regard to the mapping of forest vegetation, with a view to its assessment for commercial exploitation, the Canadian Government set an excellent example. An admirable summary of the work done, the procedures adopted and the methods of indexing the photographs was prepared by Narraway for the last Empire Forestry Conferrence in 1928 in Australia (19). It should be mentioned that the Photography is undertaken by Canadian Air Force pilots under the direction of the Survey Bureau and that the photographs are subsequently made available to anyone interested in the area they cover. The Forest Department in Burma was also a pioneer in the execution of forest surveys from the air. The results are clearly set out in Burma Forest Bulletins Nos. 11 (35) and 13, (23)†.

The photography in Burma was executed by a commercial organization for the Government and, therefore, the photographs were available for use by anyone interes-

* Unpublished communication.

† For the benefit of readers within the Empire it may be noted that these documents, together with the Proceedings of the Empire Forestry Conference in Australia, can probably be obtained on loan from the libraries of local Forest Departments.

ted in the tracts surveyed, but there has been no organized effort to use them in an ecological sense with a view to correlating vegetation with soil and geology. This failure to make the most of air photagraphs in the course of ground survey, is a common feature of many air surveys. Initially undertaken for a special purpose, the alternative uses of air survey have not been considered and, in consequence, the costs in relation to results have often been high. One body or department has borne the expenditure which should have been shared by others and which, in the ordinary course of events, would have been spread over many years. The importance of co-operation in the field of several sciences, in the work of interpreting air photographs, was specially stressed by the present writer in 1928, in Oxford Forestry Memoir No. 9, which dealt with 'Aerial Survey in relation to the development of new countries with special reference to an investigation in Northern Rhodesia' (4).

(b) *Recent developments.*

In comparison with most examples of earlier work, several recent instances of the application of air survey to scientific survey have demonstrated the growing appreciation of the importance of correlating vegetation with soil and geology in the interpretation of air photographs. It has been fully recognised that a perfect photograph is an unparalleled record of surface effect, and that its complete interpretation depends upon an approach to the question from an ecological standpoint. This point is elaborated in succeeding sections of this Communication, in the light of research undertaken by the present writer, the results of which are incorporated in a pending publication (6). Immediate reference should be made however to two of the most striking examples of similar work recorded by other workers in different parts of the Empire.

A. O. D. Mogg in South Africa (18) has recorded and illustrated with air photographs the close association of vegetation with lithological out-crops, where the underlying rock is not overlain with more than three to four feet of surface deposits. For the latter areas he has not produced a soil map, but he indicates that further differences in the vegetation can be correlated with soil, and concludes:—'Ecological plant geography may be of material assistance to the geological surveyor and soil chemist; and, as far as macroscopical geological surveys may be made from the air, the vegetation properly understood may be of considerable value in charting geological formations, as also the recording of the occurrence and course of faults'.

The other example is of such interest to soil surveyors that two extracts may be quoted. J. A. Prescott and J. K. Taylor in Australia have written (20):—During the progress of a soil survey of the Renmark irrigation settlement, considerable use has been made of aerial photographs obtained by No. 1 Squadron of the Royal

Australian Air Force during October, 1928. The close correlation between soil type and crop vigour which had been established during the survey of Block E and Ral Ral suggested that the boundaries of the soil types could be mapped to some extent from the photographic data. Complications are naturally introduced by factors such as salinity, which is not necessarily restricted to any soil type; by replantings; and, in older-established areas, by soil improvement due to drainage, the use of gypsum, and the ploughing in of green manures. In the case of the extension of the survey to the older Renmark settlement, there was the further value that no accurate maps were in existence on a scale suitable for soil survey, and the aerial photographs enabled the necessary data to be obtained for this purpose, together with an accurate record of the type, area and regularity of the cropping. The photographs were taken from a height of approximately 6,500 feet. The photographs have proved ideal as a basis for soil survey, and it was found possible accurately to fix the position of any soil boring to within two feet on the ground and to record the locality on the photograph by means of a pin prick. A close relationship was found to exist between the vegetation associations and the soil types'. And in the summary the authors say:—'Aerial photography has proved of value during the soil survey of the Renmark irrigation settlement in the revision of the existing maps, in the accurate recording of the types, areas and regularity of cropping, and in the mapping of associations of native vegetation, which have been shown to be closely correlated with established soil types'.

At this point emphasis should perhaps be laid on the established fact that the correlation of natural or semi-natural vegetation with soil is most obvious in climates such as those prevailing in the two districts just referred to, in which prolonged dry seasons are annually experienced. Striking differences in vegetation are associated with almost every soil type. With better distribution of rainfall, vegetative differences on distinct soils are less pronounced and in very humid regions may be slight. Under all but the last mentioned conditions the differences are generally clear enough to be recorded on air photographs, with the result that a map of the principal soils is indirectly furnished by the vegetation. On the other hand, if the rainfall is sufficiently heavy and well distributed to render all drained soils almost equally productive, the vegetation and the photographs tend to segregate only marshy or extremely coarse soils. In all climates the intervention of the biotic factor tends to obscure the issue and produce surface effects, both in vegetation and in other ways, which are liable to mask soil boundaries. But in long-civilized countries practical experience has frequently led to great discrimination in the use of soil and, in such circumstances, despite human intervention, the proper interpretation of surface effect on air photographs may greatly facilitate soil survey. The greatest difficuties are likely to be experienced in recently developed countries

where settlement has been rapid and haphazard. These are truths which have constantly to be borne in mind when approaching the question of air survey in relation to vegetation and soil.

Distinct problems of topographic and scientific surveyors.

If scientists in different parts of the Empire, who have had no experience of air survey, are to attempt an assessment of the local possibilities of this new weapon, it is desirable to review some of the general problems encountered in application. Initially it must be realized that the problems of geological, soil and vegetation surveyors differ in several important respects from those of topographical and cadastral surveyors and it is essential that these differences should be appreciated. If a considerable degree of accuracy is aimed at, topographical and cadastral surveyors cannot dispense altogether with control points determined either from existing maps, as is necessary over enemy territory in times of war, or by ground survey. The degree of accuracy attainable depends primarily on the number of control points, but technical excellence in photography to scale enables the air surveyor to reduce to the minimum the number of points to be fixed by the ground surveyor. With good photography, provided the differences in ground elevation are not too great (more than 1,000 feet either way from the mean), a relatively detailed topographical map can be produced on the basis of two control points to a vertical photograph. These photographs are taken with a considerable overlap in all directions and, by means of apparatus designed for the purpose, final corrections to scale can be effected on printing. When oblique photographs are taken of country intervening between two parallel lines of vertical photographs, a number of control points on each oblique are obtained by the intersection of rays drawn between prominent objects and the centre points of adjoining photographs. With oblique photographs, to convert the view obtained 'in perspective' to the correct scale 'in plan', a suitable taper grid is determined and super-printed. By this means the approximate position of topographic and cadastral features can easily be plotted on the map.

The reason that a few control points are generally sufficient for accurate topographic and cadastral mapping from air photographs clearly illustrates the principal difference between the problems of the topographic or cadastral surveyor on the one hand and the geological, soil, or vegetation surveyor on the other. Many of the details which the former set out to plot on their maps are directly recorded on the photographs, and can be recognized for what they are without further interpretation on the ground. Towns, villages, and scattered buildings; railways, streets, roads, lanes and often paths; lakes, ponds, canals, rivers and streams; quarries,

cuttings and embankments; forests, woods, spinneys and avenues; and finally, field boundaries or divisions, are all directly recorded and easily identified on air photographs. Provided the necessary corrections to scale can be effected, all these details can be transferred to a map with approximate accuracy and without further ground work. Contouring, however, may present difficulties or prove impossible, but methods have been devised by which rough contouring can often be effected as a result of stereoscopic examination of the photographs. Further improvements in this direction are to be expected. To the topographic or cadastral surveyor, therefore, technical excellence in photography to scale, though of secondary importance to the number of control points, is a very important consideration.

On the other hand, though the work of geological, soil and vegetation surveyors is obviously facilitated if photographs accurate to scale are available, perfect definition is of the first importance. The problem of these surveyors is to plot on topographical or cadastral maps either the outcrops of lithological beds, the distribution of different soils, or the boundaries of vegetative types. In many instances the desired limits cannot be transferred directly from the photographs to the map. Though many floristic and other differences in the uppermost vegetative layer are directly recorded on clear photographs, other important characters of vegetative types are obscured. Lithological and soil differences are rarely recorded directly and are reflected only by their effect on topography, vegetation, and human usage. In many parts of the world, if the vegetation has remained more or less undisturbed by man or natural agency for a considerable time, a map of the vegetation, prepared from the photographs, may closely resemble a site or soil map. But, even so, the relationship of vegetation to soil has to be proved on the ground and apparent anomalies recognised and explained. For these reasons a considerable measure of ground control is generally involved in the interpretation of surface effect. Obviously good definition is essential, but it is perhaps not so plain why accuracy to scale is often of secondary importance. This follows from the general need for considerable ground control. If much of the ground has to be covered on foot, the opportunity is afforded of checking for scale and allowing for distortion in plotting work. If a good topographical map is available, adjustments can generally be made by eye but in the absence of much topographical detail some measurements may be necessary. In the extreme case, where no topographical maps exist, only accuracy of scale in the photographs can obviate detailed survey work. But in any circumstances, the geological, soil, or vegetation surveyor is better off with than without air photographs. As already indicated, much evidence is accumulating from different parts of the world to prove this point. Special research has also been fostered by private enterprise interested in air survey, with a view to determine and demonstrate the best procedures under different conditions. The general object should always be the achieve-

ment of maximum results at a minimum cost, in terms both of money and of scientific energy. These are the matters remaining to be considered in this Communication and the factors influencing cost may first be reviewed.

Costs of air survey.

With regard to costs of air photography, much depends upon the climatic conditions, and the location and size of the area to be photographed. Climatic conditions may influence costs materially either by limiting the height from which photography can be undertaken, and therefore the area a single photograph covers, whether vertical or oblique, or by restricting the work to certain seasons of the year, or even to odd days. For instance, in parts of the British Isles, owing to the prevalence of low clouds, there may not be more than twenty flying days in the year on which extensive photography from 8,000 feet could be undertaken. From that height, unless a very wide angle lens is used, the area covered by a vertical photograph would not exceed two or three square miles. In consideration of the difficulties of organization and of the relatively small area to a photograph the cost of vertical photography is bound to be high. At the other extreme, where climatic conditions are favourable for photography at a great height (20,000 feet) over considerable periods of the year, organization is simple and some nine to fifteen square miles can be covered by a single vertical photograph. In these circumstances costs would be greatly reduced.

The location and size of the areas to be photographed would often have the greatest influence on costs. To send an aeroplane a long way to photograph a small area may be very expensive, particularly if it has then to remain idle pending suitable weather conditions. To send an aeroplane short distances to photograph small areas is much cheaper. The photography of large areas, particularly if suitable local aerodromes already exist, is comparatively cheap irrespective of location. And, if the area is large enough (200,000 square miles), the cost of establishing aerodromes may work out at a small cost per unit of area.

The difference in the costs of vertical and oblique photography depends a good deal on the conditions already mentioned. The scale of the vertical photographs is also a big consideration, but taking a scale of three to six inches to the mile, the difference is negligible if the aeroplane has to be sent any distance to photograph a small area up to 100 square miles. Where a really large area is involved the difference is enormous. If lateral and tail obliques are taken in conjunction with parallel strips of vertical photographs ten miles apart, an area could be photographed in at least one-sixth of the flying time involved with complete vertical

photography. Moreover, the actual number of exposures necessary would be greatly reduced.

Since so many factors may influence costs, it would be misleading to quote average figures, but some idea of the two extremes may be given for small scale photography. Under very adverse conditions such work, either with vertical or oblique photography, may cost as much as £20 to the square mile. Clearly this is an uneconomic figure except under very special circumstances. Under peculiarly favourable conditions a judicious combination of vertical and oblique photography may cost no more than two or three shillings to the square mile. These figures do not include the costs of preparing topographic maps. They merely allow for producing a set of photographs covering the area. But the latter figure indicates that the cost of supplying scientists with vertical and oblique photographs of the ground over which they have to work may not be prohibitive. If the photographs are taken primarily for topographic purposes, the costs will be shared. In any circumstances scientists should seek to adapt their methods of working with a view to the maximum economy possible without loss in efficiency. To appreciate what may be done in this respect, consideration must be given to the use of photographs in the field and the results to be expected.

The air view.

With regard to the use of air photographs for scientific purposes, reference may first be made to the view obtainable from the air by visual observation. This view differs from that supplied by photographs in that it is wider and is enhanced by colour. On the photographs, different colours appear as shades and tones of white, grey and black. On the ground, with the corresponding photograph in hand the various shades are easily interpreted, but in the office they can be very misleading. Much depends on the filter used in photography. In general, browns and reds tend to turn out white or light grey, and greens or blues appear as various shades of grey to black. Shadows of trees are also black and the reflection of light by water or bare wet soils produces a curious white effect. From the air, colours are very distinct and of great significance in the interpretation of surface effects. Apart from colour differences, the observer obtains a general view of large tracts of country. The extent of the view depends partly on the height and partly on the visibility ground, mists or haze often obscuring the ground almost as much as clouds. With a clear atmosphere the best definition is obtained with the sun behind or to one side. When flying into the wind to reduce the ground speed and at an elevation of 6,000 feet or more, the country beneath and towards the horizon passes slowly by.

With good visibility prominent features may be located to a distance of thirty or forty miles. If they are looked for, many details may be identified, up to six or seven miles, but the tendency at a considerable height (8,000 feet or more) is to lose sight of detail and to see only the general surface effects. This last fact alone is of great practical importance and, therefore, the significance of what has been termed the 'air view' may be emphasized.

Regional survey from the air.

If a competent observer from the air has studied the principles of regional survey and learnt on the ground to appreciate what may constitute a unit natural region of country, he will normally find that he can recognize from the air and plot the approximate boundaries of these regional units on a topographical map. Literally he is able to achieve at a glance from the air a result which the regional surveyor on the ground generally considers as one of the ultimate objects of detailed field investigations. Unit regions may be large or small, but invariably they are characterized by peculiar combinations of climate, lithology, topography, and soils, and are constituted by distinctive sets of sites, or units of ground. In regions of mature topography, sites and soils are generally coincident in extent, but, in hilly or broken country, slope and local climate may occasionally necessitate the subdivision of a soil into two or more sites. As already mentioned, if the vegetation is natural or semi-natural, the differences in soil or site within a region are generally reflected by the vegetative types, and the relative distribution of sites will thus indirectly give a characteristic surface effect to the region as a whole. If man has occupied and cultivated the area for any length of time, his scientific though empirical usage of site, combined with any natural differences in the vegetation, may still reflect the site differences and produce an effect characteristic of the region as a whole. But wherever inexperience or economic factors have intervened to permit or force human usage of sites contrary to the scientific principles of agriculture or forestry, vegetation and other surface effects may obscure natural boundaries. Thus, in regions of mature topography, the facility with which unit natural regions can be distinguished from the air is a fair measure of the extent of human influence on the one hand and of the degree to which it is scientific on the other. Though reference has only been made to the recognition of unit natural regions in the course of visual observation from the air, differences in colour being of great assistance in this matter, experience has shown that regional boundaries can generally be traced on air photographs. In this respect oblique photographs are particularly useful for the simple reason that they give such a wide view.

The recognition and delimitation of the unit natural regions into which a countryside is divided is of practical importance in various ways. In general these regions should be the units considered in framing and applying a policy of land settlement. Once settled they are the units within which scientific, economic, and social researches should be repeated with a view to the subsequent local application of the results. If large enough, therefore, they are suitable units for dissemination of knowledge among the users of the land. With regard to scientific survey in particular, a preliminary recognition of unit regions is invaluable, more especially where the work extends over large areas. The problem of efficient sampling becomes easy of solution. All experienced lithological, soil and vegetation surveyors realize that the issue of their work depends upon the intensity of their initial studies. In a sense they always resort to sampling and obviously, if they know in advance the best locations for intensive study and the limits within which the results apply, they can restrict their detailed observations to relatively small areas without loss of efficiency. Their object at this stage is to determine the different lithological beds, soils and vegetative types to be mapped and the system or systems under which they are likely to be distributed. If this is first accomplished and the several relationships between lithology, topography, soils and vegetation are established, subsequent mapping in the region as a whole is a comparatively simple proposition. This fact is well illustrated by reference to the problem of interpreting scientific phenomena on air photographs. If one or two sample photographs of a region are interpreted by intensive study on the ground, the correct interpretation of other photographs of the same region can be achieved with a minimum of ground control. If a photograph of an adjoining region which has not been visited is studied, the interpreter will at once be at a loss to understand much of what the photograph records. Clearly preliminary knowledge of regional limits, and a general appreciation of the differences in surface effect to be encountered within a region, enable a surveyor to direct his field operations with a view to extracting the maximum results with the minimum of energy. Thus the air view and oblique photographs, possibly obtainable without recourse to an organized air survey, open up possibilities of economy in scientific survey which should be widely explored.

Procedure in visual reconnaissance.

To obtain the best results an observer from the air should adopt a definite procedure. Working with a topographical base map, on which too many details are undesirable, *he should concentrate all the time on reading the ground in relation to the map*, fixing the positions of the objects under observation by reference to such

features as towns, villages, rivers, streams, roads and railways. If he is successful in this, he will find little difficulty in sketching the approximate position of regional boundaries. If he is not certain about some of these, he should at least be able to distinguish and map areas under grass from areas under crops and agricultural land from wasteland or forest. An observer may be able to map much more, but there is a limit to what he can achieve in the time available and undoubtedly in this work the personal equation is a big factor. Some men can train themselves to map much more than others, even to delimiting, here and there, quite minor differences in vegetation and soil. But the fact that the best of observers has not the time to map every difference he can see is a very strong argument in favour of oblique photography.

Oblique photography.

Oblique photographs can be taken from many types of aeroplane not specially designed for air photography. The two requirements are an unrestricted lateral view from one of the cockpits and a suitable camera. The latter is essential if good definition is to be obtained. It must be realized that the camera has to be exposed in the slip-stream of the propeller and that one with bellows or an unsheltered lens is useless. To avoid blurring due to vibration, a fast shutter capable of speeds up to one-thousandth of a second is desirable, but with care longer exposures can with advantage be given. A filter is necessary to differentiate colours clearly and to pierce hazes almost imperceptible to the human eye. Special panchromatic plates or films should be used and to ensure that the camera is held horizontal, either the true or haze horizon should be included within the sights at the moment of exposure. The best speed and stop to use can only be determined by experience, but with a suitable camera and some trial, a reasonable proportion of good photographs should be obtained, however much they vary in scale, such photographs can be used to check regional and other units sketched on the topographical map and allow of filling in many details which are not easily sketched from the air. In the absence of an organized air survey and, in consequence, provision of vertical photographs, these obliques can also be used as rough maps in the field. But, for these purposes, technical perfection in vertical and oblique photography to scale is certainly to be preferred and therefore, wherever possible, the services of a professional air surveyor should be enlisted.

Organization of air survey for scientific purposes.

If an organized survey is possible, a regional reconnaissance should first be undertaken. This will help to determine the best orientation of the lines of flight

for purposes of photography. The general idea would be to take vertical photographs on parallel flights in conjunction with oblique photography of the intervening areas. Normally the lines of vertical photographs should be at right angles to the principal drainage systems of the areas under survey. In these circumstances, some vertical photographs will usually be obtained of each unit natural region in the district.

Following upon photography, a scientific surveyor should draw up his general plan of campaign, selecting photographs in each region for purposes of detailed interpretation on the ground. At this stage of the work the closest possible co-operation is desirable between geologists, pedologists, and ecologists. This applies with considerable force whether air photographs are available or not, but as previously indicated, with a photograph the record is largely in terms of surface effect, and in the absence of an ecologist, a geological or soil surveyor may have the greatest difficulty in the work of interpretation. However, the co-operation of an ecologist may not be possible and therefore it is desirable to outline the general procedure to be adopted when using air photographs for purposes of soil survey in the field.

Use of air photographs in the field.

If the photographs have not been corrected for scale, a topographical base map of the area is essential and the surveyor must be prepared to carry out the measurements necessary for the correction of distortion. If overlapping vertical photographs approximately to scale are available, they should certainly be examined in a stereoscope before starting out. In this way the ground relief can be studied and a good idea of the 'terrain' obtained in advance. With such photographs the topographic map may be dispensed with and xylonite sheeting, which is extremely transparent, used for plotting work and indexing notes recorded in the field. With any type of photograph it is a good plan before going into the field to outline with dots in white photographer's ink all the differences which can be detected in surface effects. This ink, unlike all others, will subsequently wash off without spoiling the photograph. On entering the field the surveyor should set out to check the significance of all differences in surface effect. As he proceeds, he should transfer to topographical map or xylonite sheeting the limits of lithological outcrops and soils, and, if the circumstances so suggest, he should sub-divide the soil into sites. At this stage of intensive sampling he should simply use the photographs as indicators of surface differences and as an aid to mapping. His aim should be to determine how many different soils or sites go to constitute the region and their relative distribution one to another. If he completes the soil or site map of the area covered by a sample photographic record of the vegetative differences, he should

have reached a reasonable understanding of the region as a whole and be in a position to say how far differences in the vegetation, whether natural or artificial, reflect the soils or sites. He may well have realized that differences in growth-form and luxuriance of the vegetation are as significant indicators of soils or sites as differences in floristics. Indeed, if man has largely disturbed the natural vegetation, he may often find that some of the most obvious differences in floristics are of little significance.

Regional interpretation of air photographs.

With the knowledge gained in sampling, the surveyor should turn to an interpretation of other photographs of the same region. If he has previously viewed the region as a whole from the air and carried out any sketching of surface effects, this work should also be taken into consideration. The surveyor may find that the differences in surface effect are often sufficiently clear for him to identify and plot many of the boundaries of the soils and sites which constitute the region. His map would rarely be completed by this means, but he can see at a glance exactly how he had best traverse the area on the ground to check and complete the mapping. Proceeding into the field he should again use the photographs merely as indicators of surface differences and as an aid to mapping. It is quite possible that the surveyor's attention will be drawn to differences which reflect one or two soils and sites not found on the area originally sampled. In that eventuality local investigation may enable him to fill up several of the remaining gaps in his map, subject only to local check. In the application of this procedure experience has shown that a great saving in time is effected and that the results are at least as good and generally better than those achieved in other ways. Obviously, when dealing with large areas, the completion of mapping within regions may have to be spread over a considerable period of time. In these circumstances regions may be dealt with in their relative order of importance and at a rate dictated by the staff available. But completion of mapping may be urgent and it is, therefore, important to remember that ground control at this stage can be greatly reduced without loss of accuracy by employing photographs to scale.

Summary.

To sum up, the air view and air photography can assist the scientific surveyor in three stages of his work—in the preliminary reconnaissance, in the intensive sampling, and in the completion of mapping. The early knowledge gained about the unit natural regions is generally of incalculable value. Subject to the personal equation in the air and the possibility of taking good oblique photographs, an individual surveyor may be able to dispense at this stage with the specialist air

photographer. In the second stage of intensive sampling, a surveyor has to traverse the sample area so intensively that photographs accurate to scale are not necessary, however desirable. But for this purpose vertical photographs are infinitely to be preferred to obliques and in most cases special aeroplanes and cameras would be necessary. Again, in the last stage, if the preparation of maps is urgent and the survey staff is limited, perfect photographs approximately to scale are essential. In both these latter stages the co-operation of the specialist air surveyor is normally involved. Clearly the means by which the air view and air photography can best be brought to bear on the problems of the scientific surveyor must largely depend upon local conditions. The great need of the moment is for scientists in different parts of the Empire to assess the possibilities of application in their own and neighbouring districts. If this communication helps them to appreciate these possibilities at their true worth it will have served its purpose. The reader will have realized that apart from the general review of the history and problems of air survey, directions have been furnished as to the tests which should be applied wherever facilities are available for getting into the air or for working with air photographs, and that an outline has been given of the general procedure to be adopted with a view to achieving the maximum results at the minimum cost.

BIBLIOGRAPHY.

(1) ANDREWS, L. A.—
1921. Fire protection and survey by aeroplane. *Can. For. Mag.*, Vol. XVII, pp. 419—422, 451—452, 461—462.

(2) BARRINGTON, A. H. M.—
1925. Air Survey. Administration Report, Working Plans Circle, Burma, pp. 1—6.

(3) BLANDFORD, H. R.—
1924. Aero-photo survey and mapping of forests of Irawaddy Delta. *Indian Forester*, Vol. 50, pp. 605—616.

(4) BOURNE, R.—
1928. Air Survey in relation to the development of new countries with special reference to an investigation carried out in Northern Rhodesia. Oxford Forestry Memoir No. 9, pp. 35.

(5) BOURNE, R.—
1930. Air Survey within the Empire; a summary of the general conclusions reached in recent research. *The Air Annual of the British Empire*, Vol. II. pp. 15—21.

(6) BOURNE, R.—
1931. Regional Survey and its relation to Stock-taking of the Empire's Agricultural and Forest Resources. (Illustrated by Air Photographs.) Oxford Forestry Memoir, in press.

(7) CHEVALIER, A.—
1930. Aviation in the service of tropical agriculture and botanical geography. *Rev. Bot. Appl. Agr. Trop. Bull.* 106, pp. 353—356.

(8) COCHRAN PATRIC, C. K.—
1929. Air Survey and Air Photography. *The Air Annual of the British Empire*, Vol. I, pp. 257—267.
(9) CROSTHWAIT, H. L.—
1928. Air Survey and Empire Development. *Jour. Roy. Soc. Arts.*, Vol. LXXVII. pp. 161—182.
(10) ESCHERICH, K.—
1929. Die Flugzeugbestauberung gegen Forstschadling. No. 12, Flugschrift d.d. Ges. f. angew. Ent., pp. 60.
(11) JENKINS, F. T.—
1924. Aircraft in Forest Surveys. *Can. For. Mag.* Vol. XX, pp. 388—390, 456—458.
(12) JENKINS, F. T.—
1925. Aerial Forest Mapping. *Can. For. Mag.* Vol. XXI, pp. 265—266.
(13) JENKINS, F. T.—
1926. Aerial sketching of forest areas. Report on Civil Aviation, Canada. pp. 110.
(14) JONES & GRIFFITHS—
1929. Aerial Surveying. Cambridge. pp. 159.
(15) KRUTSCH—
1925. Das Luftbild im Dienste der Forsteinrichtung. *Tharandt. Forst. Jahrb.*, Vol. 76, pp. 97—150.
(16) LEE, W. T.—
1922. The face of the Earth as seen from the Air. Am. Geog. Soc. Spec. Publn. No. 4, pp. 110.
(17) MCKINLEY, A. C.—
1929. Applied Aerial Photography. New York, pp. 341.
(18) MOGG, A. O. D.—
1929. A preliminary account of the Flora of Pretoria in relation to the Geology. Compte Rendu, XV Inter. Geol. Congress, S. Africa, Vol. II, 1929, pp. 651—669.
(19) NARRAWAY, A. M.—
1928. Aerial Surveys in Canada. Proceedings of the Empire Forestry Conference, Australia, 1928. pp. 759—763.
(20) PRESCOTT & TAYLOR—
1930. The value of Aerial Photography in relation to Soil Surveys and Classification. *Jour. Council Sci. and Ind. Res.*, Commonwealth of Australia. pp. 229—230.
(21) ROBBINS, C. R.—
1929. Air Survey and Forestry. *Emp. For. Jour.* pp. 205—228.
(22) ROBBINS, C. R.—
1931. An economic aspect of regional survey. *Jour. Eccl.* Vol. XIX, No. 1, pp. 25—33.
(23) SCOTT, C. W. & ROBBINS, C. R.—
1926. Report on aerial reconnaissance of Tavoy and Mergui District. *Burma For. Bull.* No. 13, pp. 76.
(24) THELEN, R.—
1919. Aerial photography and national forest mapping. *Am. Jour. For.* Vol. XVII, pp. 515—522.
(25) THOMAS, H. H.—
1920. Air-craft photography in the service of science. *Nature*, Vol. CV, pp. 457—459.

(26) THOMAS, H. H.—
1920. Geographical reconnaissance by aeroplane photography, with special reference to the work done on the Palestine front. *Geog. Jour.* Vol. 55, p. 349.

(27) VOLMAT, J.—
1919. Application de la photographic aerienne aux leves hydrographiques. Comptes Rendus de l'Acad. des Sciences, Paris. Vol. 169, p. 717.

(28) VOLMAT, J.—
1919. Rapport sur la mission photo-hydrographique de Brest, Annales Hydrographiques.

(29) WILSON, E.—
1920. Use of sea-planes in forest mapping. *Am. Jour. For.* Vol. XVIII, pp. 1—5.

(30) WILSON, E.—
1922. Forest mapping from aerial photographs. *Am. Jour. For.* Vol. XX, pp. 113—116.

(31) WILSON, E.—
1926. Aerial Survey and forest conditions. Proc. First Inter. Silvic. Congress, Vol. III, pp. 222—237.

(32) WILSON, J. A.—
1921. Flying services rendered to Canada's forests. *Can. For. Mag.* Vol. XVII, pp. 525—527.

(33) Aerial mapping. 1922. Administration Report, Department of Lands and Forests, Ontario, pp. 222—233.

(34) Aerial Survey of James Bay Region, Ontario, 1923. *Can. For. Mag.* Vol. XIX, pp. 738—741.

(35) Aero-photo Survey, Irawaddy, 1925. *Burma For. Bull.* No. 11, pp. 43.

(36) Air reconnaissance of South Tenasserim. 1925. *Indian Forester*, Vol. 51, pp. 593—599.

(37) Report on Civil Aviation, Canada, 1924, pp. 110.

RECENT LITERATURE ON HERBAGE PLANTS AND FODDER CROPS.

The Imperial Bureau of Plant Genetics Herbage Plants (Aberystwyth, Wales) has recently issued the following publications :—

Herbage Abstracts 1931 (Vol. I, No. 1) June 1931.

Bulletin No. 3.—The breeding of Herbage Plants : Technique adopted at the Welsh Plant Breeding Station. (Price 3 *s.*)

Bulletin No. 4.—Abstract Review of Lucerne Literature during the Period 1925-30. (Price 2-6.)

Bulletin No. 5.—Research in progress on Herbage Plants, Forage Crops and General Grassland problems in the British Empire. (Price 2-6.)

Herbage Abstracts, as its name implies, contains abstracts of recent literature on grassland and fodder crop problems, including the many aspects of pasture land management and improvement, hay production and the raising of special fodder crops.

Bulletin No. 3 describes the technique in use at Aberystwyth which has been worked out by Professor Stapledon and his colleagues, especially Mr. Jenkin (Grasses) and Mr. Williams (Clovers and Lucerne). The results of the work quoted are already classical, but rarely has so full a description of a new technique been made available to other workers. Professor Stapledon's own concluding remarks will illustrate the outlook of the bulletin :—

" In Science as in Art, a technique in the last resort should be judged by one standard only—is it successful ? That is to say, in pure science does it or does it not lead to the collection of data elucidatory of one's problem ?

In applied science it is still necessary to acquire data elucidatory of all aspects of one's problem, but it is eminently desirable to do more than this. If a problem is in fact of real economic concern it is of the first importance to find a solution, as quickly and as cheaply as possible.

In the realm of herbage breeding by no means the easiest part of the problem is to ascertain with reasonable assurance precisely to what ends it is desirable to

breed. The actual breeding work must inevitably entail detailed researches—must inevitably demand considerable resources, and to be carried to a successful issue must take no mean period of time.

Before critical breeding work on any particular term of reference is embarked upon, it is therefore very desirable to have fully explored the agrostological potentialities of the species taken in hand. This is of course to ensure that one is really trying to breed in sympathy with an urgent economic need. It was primarily with a view to this end that I started my "breeding" work on cocksfoot by the adoption of rough and ready methods. The aggregate strains I early acquired proved invaluable for sward trials and yielded important information as to the trend of sward behaviour of sharply contrasting types: information in fact which has proved its worth in relation to the whole problem of herbage breeding.

When assembling material in order to begin studies on any particular species it is a relatively simple matter to organise a certain amount of group breeding. The seed so obtained will in any event be essential for preliminary sward trials. While if some of the material so "bred" proves to be relatively true to a desirable type, it may be well worth growing it on and actually marketing it as a stop-gap product, pending the production of a greatly improved strain of reasonable genetical purity.

Since in the case of nearly all herbage species improvement by breeding has been practically un-tried, the field is an exceptionally wide one. In many instances it follows, therefore, that important improvements are likely in the first instance to be possible by the adoption of comparatively simple methods. Ultimately, critical methods, such as are already abundantly called for in the case of the more generally used grasses and clovers, will have to be applied to all species having real economic significance.

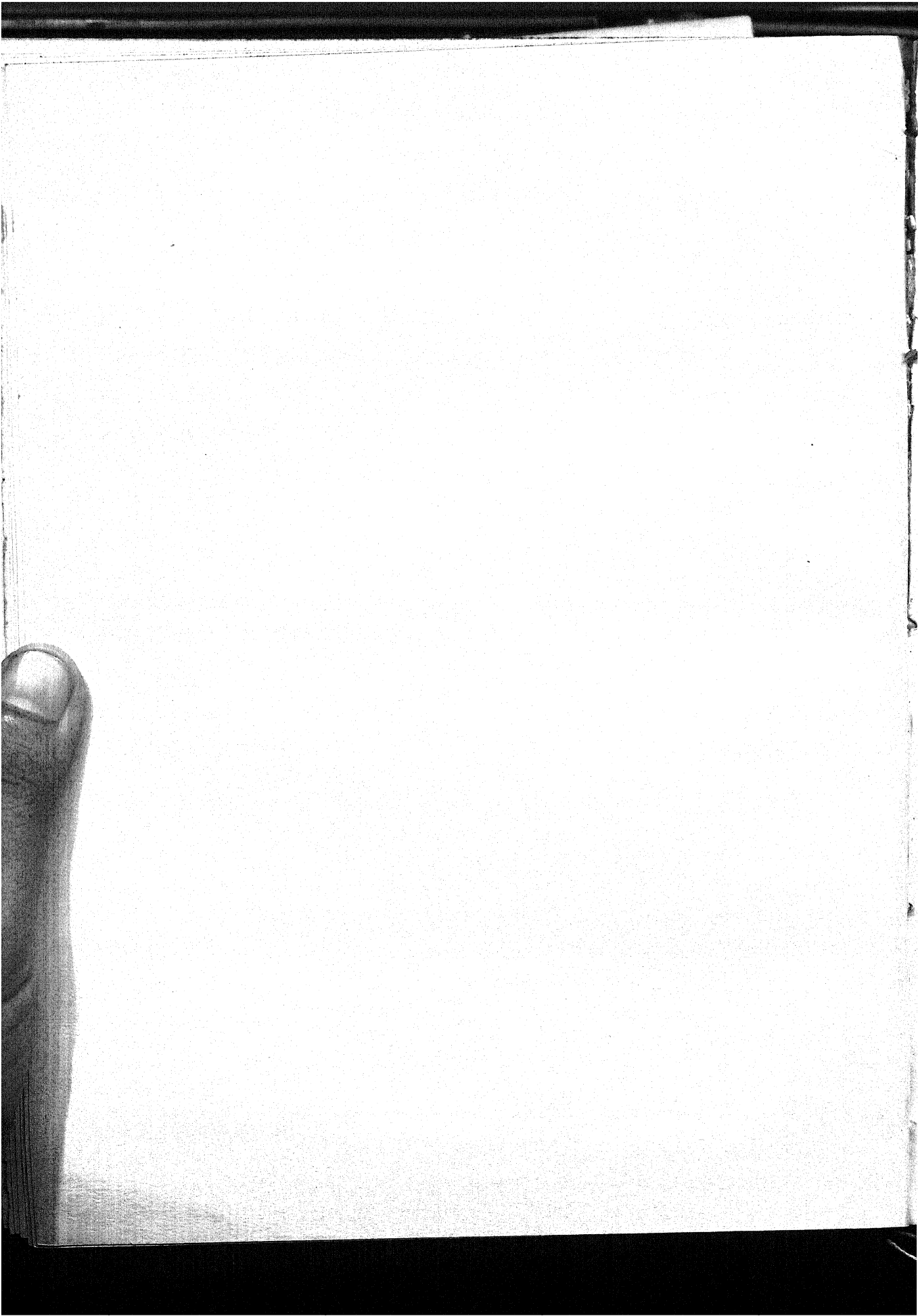
In conclusion, it does seem desirable to emphasise the necessity of basing critical genetical breeding work on a solid foundation built of well-planned investigations on the grasslands to be catered for and the relation of one's species to these grasslands. In this connection it is worthy of remark that all of us who are concerned with herbage breeding at Aberystwyth have served a considerable apprenticeship to 'general grassland inveetigations'.

It is my firm belief that in the realm of herbage improvement by breeding we shall only reap the full harvest of what is undoubtedly possible by the heartiest co-operation of the ecologist and the geneticist."

Mr. Williams' work on the breeding of clover and lucerne should be studied by all engaged on the improvement of leguminous crops in many species of which self-sterility (in varying degree) presents special difficulties to the plant breeder.

Bulletin No. 4 brings together in convenient form abstracts of the recent literature on the important lucerne crop. Future issues of *Herbage Abstracts* will contain abstracts of new literature.

Bulletin No. 5 has been issued by the Bureau with the object of giving agriculturalists and research workers throughout the Empire some indication of what work is being carried on elsewhere on herbage problems. Brief as the summaries must necessarily be, a surprising amount of detailed information is conveyed in a small space. Suggestions and criticisms are welcomed by the Bureau and any additions or amendments to the Indian Section of the Bulletin, or proposals for the inclusion of additional information in future editions of the bulletins, may be forwarded to the Agricultural Expert, Imperial Council of Agricultural Research, New Delhi.



ORIGINAL ARTICLES

A TITRATION METHOD FOR DETERMINING THE TOTAL AND EXCHANGEABLE BASES IN SOILS.

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INTRODUCTORY.

In connection with certain investigations in progress in this laboratory it was necessary to determine the total exchangeable bases in a large number of calcareous soils. Although many methods have been used successfully for acidic and neutral soils, none of them could be used satisfactorily for these Burma soils. The unsuitability of the ammonium chloride method of Gedroiz [1918] and of Kelley and Brown [1924] for calcareous soils has been pointed out by Wilson [1928] and Burgess [1929]. Hissink [1923, 1925] used sodium chloride as the replacing agent for soils containing carbonates and applied a correction for the calcium carbonate dissolved by the reagent on the assumption that the calcium contained in the second litre of leachate corresponds to the solubility of calcium carbonate. This assumption has been shown to be unreliable by the work of Tyurin [1927] and Turner [1928]. On the other hand, in an examination of ten highly calcareous soils (15-40 per cent. $CaCO_3$) by Hissink's method Menchikowsky and Ravikovitch [1929] found a constant value for the calcium dissolved by the second litre of normal sodium chloride, but with soils low in carbonates (less than 0·5 per cent. $CaCO_3$) Ravikovitch [1930] found no such constancy. In the latter case the correction for the dissolved $CaCO_3$ by the titration method of Tyurin agreed closely with the CO_2 estimation method of Gedroiz, but the values thus determined for the correction were about 4-5 times those obtained by Hissink's method.

In order to avoid the difficulties mentioned above, Burgess [1929] has developed the use of an alcoholic solution of barium chloride for base exchange work with all kinds of soils. The solubility of $CaCO_3$ in this reagent is only 14 parts per million and therefore Burgess recommends that the correction may be neglected. He finds that magnesium carbonate (basic) is very much more soluble, but as there is evidence (under investigation) that the $MgCO_3$ of soils differs from $CaCO_3$, presumably in being much less soluble, he does not consider that a correction

is necessary for the dissolved magnesium carbonate. Kelley [1929] has raised an objection to the use of barium chloride or any other salt of a divalent metal as these would react with compounds such as calcium carbonate and calcium metasilicate and dissolve the calcium which would be erroneously classed as exchangeable. Similar errors are liable to be caused by reaction with the magnesium compounds also. According to McIntire and Shaw [1930] normal magnesium carbonate is readily hydrolysed in the soil under humid conditions into magnesium bicarbonate and the basic carbonate $3MgCO_3 . Mg(OH)_2$, the latter undergoing further hydrolysis into hydroxide but more slowly. The exchange complex of the soil is therefore able to fix magnesium more readily than calcium, if both are added as carbonates to the soil. However, under arid or semi-arid conditions $MgCO_3$ (basic) may be expected to persist in the soil due to periodical deposition from the bicarbonate contained in the soil solution. Kelley [1917] has also shown that magnesium carbonate undergoes extensive silication in the soil. Therefore it seems to be fairly obvious that the error due to double decomposition of non-exchangeable magnesium compounds with barium chloride or other similar leaching agent will not be negligible.

Another reagent that has been used for base exchange work is a normal solution of neutral ammonium acetate investigated by Schollenburger [1927] who has pointed out its special merits. When applied to calcareous soils a correction was applied by Schollenburger and Dreibelbis [1930] to the calcium dissolved from the carbonate by the Gedroiz method of determining the CO_2 in the soil before and after leaching and assuming it to be entirely derived from $CaCO_3$. Ravikovitch [1930] made a similar assumption but the soils examined by him contained only very small amounts of magnesium. Wilson [1930] studied the exchangeable calcium in soils under different treatments and assumed that the whole of the carbonate in the soil was associated with calcium, not only because of the small amounts of magnesium present but also because of certain other observations about which data have been promised. It may be stated here that in connection with a soil survey of the Mandalay Canal area in this laboratory a highly significant correlation of +·8 has been obtained for the association of CO_2 with the ratio CaO/MgO.

The use of the chlorides of ammonium, sodium or barium, or ammonium acetate to determine the replaceable bases in calcareous soils therefore involves (i) the partial solution of calcium carbonate which prevents the rapid and complete removal of the exchangeable calcium from the soil, the end point being uncertain; (ii) the determination of CO_2 in the soil twice to get the correction; and (iii) the determination of the bases separately to obtain the total. It would therefore be advantageous to employ a reagent which dissolves the whole of the carbonates in

the soil and apply a correction for these which would require only a single CO_2 determination. If the reagent enables a titration method to be used for the total bases it would be a further advantage.

Bradfield [1927] suggested the titration of the alkaline soil extract in connection with the electrodialysis method for determining replaceable bases in soils. Electrodialysis of soil has been extensively studied, notably by Mattson [1926], Humfeld and Alben [1927], Crowther and Basu [1929] and Wilson [1928-1929]. When applied to calcareous soils this method is generally not satisfactory owing to a partial decomposition of the carbonate. Wilson [1929] showed that replaceable calcium was completely removed from non-calcareous soils by electrodialysis for 8 hours. He assumed [1930] that in calcareous soils also the whole of the replaceable calcium would be removed in 8 hours and corrected for the calcium dissolved from the carbonate by CO_2 estimation. However, Humfeld and Alben [1927] seem to have obtained constant titration values for calcareous soils by prolonged electrodialysis, indicating complete solution of the carbonates.

An elegant titration method for determining the total bases in non-calcareous soils has been devised by Rice-Williams [1929] who used acetic acid as the leaching agent, the leachate being converted into oxides or carbonates by evaporation and ignition.

Bray and Willhite [1929] recommend ammonium acetate in an essentially similar method.

Spurway [1925] and Kappen [1929] suggested that the total bases in acid and neutral soils may be determined with sufficient accuracy by shaking the soil with standard acid and titrating the residual acidity with standard alkali. Belgrave [1927] tested this method on the acid organic soils of Malaya, which are extremely poor in bases and found two sources of error, *viz.*, (1) adsorption of the chloride ion by the soil which caused a reduction in acidity in the extract, and (2) extraction of organic matter from the soil which caused an increased acidity, sometimes even giving highly negative results. Therefore Belgrave used 0·05 $N AlCl_3$ instead of HCl and obtained satisfactory results, which agreed with the barium chloride leaching method. When the base content of the soil was less than 3 milliequivalents per cent., Belgrave corrected for the adsorption effect by titrating the chloride ion and the acidity in the extract and deducting the equivalent value of the adsorbed chloride from the apparent value of the total bases corresponding to the acidity. Apart from the effect of the organic matter, Mattson [1927] has shown that chloride ions are positively adsorbed from acid solutions by soils with a low silica-sesquioxide ratio and negatively adsorbed from soils with a high ratio. A leaching method would, of course, be free from these objections.

Recently Puri [1931] has published a method for determining the replaceable bases in soils. According to this 10 grams of soil are treated successively with 0·5 *N* HCl and 0·1 *N* HCl and shaken on a shaking machine for 2 hours. The soil is next thrown on a Buchner funnel and washed four times with 100 c.c. portions of 0·05 *N* HCl and finally twice with 100 c.c. portions of water. The filtrate is made up to volume and the residual acid in an aliquot portion of it titrated with alkali using phenolphthalein as indicator, no specific conditions being laid down for the titration. From this titration the bases dissolved from the soil (exchangeable and carbonate) can be calculated. The CO_2 in the original soil is determined by a method [1930] also devised by him and the equivalent base value subtracted from the total bases. The chief difficulties of this method are (*i*) the necessity to stock large quantities of standardised acids of three different strengths and (*ii*) the accurate measurement of large volumes. Puri has compared the values of the exchangeable calcium extracted from a calcareous soil, P. C. 2 containing 5 per cent. $CaCO_3$, by various reagents, his values being 160·7 m. e. with *N* NH_4Cl, 133·0 m. e. with 0·1 *N* NH_4Cl and 64·4 m. e. with 0·1 *N* $BaCl_2$ (alcoholic). The value for total bases by his method is 181·8 m. e. and for exchangeable bases 81·8 m. e. From the figures Puri concludes that ammonium chloride is unsuitable for base exchange work. Obviously he has not corrected for the dissolved $CaCO_3$ in the figures quoted except in those obtained by his method. If the correction is applied to the value of calcium extracted by *N* NH_4Cl the agreement seems to be satisfactory.

It is the purpose of this paper to present a method for the estimation of the exchangeable bases in soils which is rapid and reasonably accurate.

Outline of the Author's method.

Briefly the method consists in (*i*) leaching the soil with dilute HCl (approximately 0·05 *N*) until the bases are removed, (*ii*) making up the volume to one litre and titrating the acidity in an aliquot portion with standard alkali using phenolphthalein as indicator, (*iii*) titrating the same portion with standard $AgNO_3$ to obtain the total chloride content, and (*iv*) determining the CO_2 in the original soil by a standard method.

Since the soil extract contains HCl and the whole of the bases as chlorides (assuming the original soil to be chloride-free) the bases correspond to the difference in titration values for the chloride content and the acidity of the extract. The carbonates may be subtracted from the total bases to obtain the exchangeable bases.

The principle involved in this method can be applied to other reagents also. In the acetic acid method of Rice-Williams the carbonates and replaceable bases

may be determined together in the leachate by titration and the carbonates in the original soil corrected for. Formic acid or nitric acid may be similarly used because the acidity can be titrated and the negative radical, formate or nitrate, estimated also by titration. As the chloride ion is easy to titrate HCl is a very convenient leaching agent.

Experimental.

The following solutions are required :—

(*i*) A solution of HCl containing 5 c.c. of conc. acid per litre. This is approximately 0·05 *N* but need not be standardised. This solution may be prepared in a Winchester quart bottle as often as required.

(*ii*) A decinormal solution of NaOH which has been freed from carbonate by treatment with baryta or by some other method. A saturated solution of calcium hydroxide may be used instead. It must be freshly titrated before use. The fresh solution is about 0·04 *N*. In the experiments described in this paper lime water was used to demonstrate the differences due to various indicators which would not be so striking when stronger solutions of alkali were used.

(*iii*) A decinormal solution of silver nitrate.

(*iv*) A ten per cent. solution of pure potassium chromate or one per cent. solution of fluorescein.

The soils used were taken from the permanent manurial plots of the College Farm, air-dried and passed through a 2 mm. sieve. They contain practically no chlorides. The organic matter was low, being less than three per cent. humus. The soils were only slightly calcareous, the $CaCO_3$ being less than one per cent. Qualitative tests showed that the extracts contained only the merest traces of sulphate and phosphate. In any case they will not interfere with the titration.

25 grms. of soil were weighed into a tall narrow beaker and treated with 75 c.c. of dilute HCl. The mixture was stirred at short intervals, allowed to settle and the clear solution decanted through a filter into a measuring flask of 1 litre capacity. The treatment was repeated using 60—70 c.c. of acid each time. The calcium was usually completely removed when about 600 c.c. of liquid had been collected in the flask. According to Gedroiz the energy of absorption of calcium by soil is greater than that of magnesium, potassium and sodium, but Joseph and Oakley [1929] and also Wilson [1929] have shown that potassium is more strongly absorbed by soil than Ca, Mg and Na. As Ca is very much more abundant in the soil than the other bases it may be assumed that when Ca is entirely removed the other bases are also removed. Accordingly when the test had shown the complete removal of Ca, three or four more treatments were repeated as a

precaution and then the volume was made up to 1 litre. Several samples were treated simultaneously. With soils poor in bases 50 grms. were used but with highly calcareous soils 10 grms. only were used, and in the latter case the strength of the leaching acid was increased to 0·1 *N* or even 0·2 *N* in the earlier stages of the treatment so as to keep the volume of leachate below one litre.

Fifty c.c. of the leachate was titrated with 0·1 *N* NaOH (CO_2-free) using phenolphthalein as indicator until the colour remained just pink on boiling. When the end point was approached a pale pink colour developed, but on boiling the colour disappeared, and the liquid remained colourless on cooling. Further drops of alkali were added and the boiling repeated. At the end point the pink colour persisted even on boiling, although very pale. On cooling the colour deepened.

The neutral solution obtained as above was thoroughly cooled and the pink colour discharged by adding a small drop of dilute acetic acid. A fresh portion of the extract neutralised with a slight excess of pure $CaCO_3$ may be used more conveniently. Two drops of potassium chromate or fluorescein were then added and the solution titrated for chloride with silver nitrate. A blank correction was made in each case. Fluorescein is better than chromate as the end point is sharper and moreover it may be used in presence of barium.

If 50 c.c. of soil extract required 24 c.c. of 0·1 *N* NaOH, and 29 c.c. of 0·1 *N* Ag NO_3, the total bases correspond to 5 c.c. of 0·1 *N* solution. Hence the total bases in 100 grms. of soil are equal to $\frac{5}{10} \times \frac{1000}{50} \times \frac{100}{25}$ or 40 milli-eqts. An error of one drop (0·05 c.c.) in the titration difference will amount to 0·4 milli-eqts. when 25 grms. of soil are leached to 1 litre and 50 c.c. titrated.

The indicator.

In the titration for acidity the indicator used requires comment. Various indicators have been used for the titration of soil extracts but data are not given to show which of the bases are returned as replaceable when a particular indicator is used. The usual idea has been that the chlorides of iron (ic) and aluminium are so readily hydrolysed that their solutions may be regarded as dilute HCl and that any ordinary indicator may be used in titrating them. Thus, for the titration of soil extracts obtained by electrodialysis methyl red has been recommended by Humfeld [1928], Wilson [1928], Crowther and Basu [1929] and Basu [1931]. In particular Humfeld states that when hydroxides are titrated directly with standard HCl using methyl red as indicator the end point is not sharp due to the absorption of the indicator by the hydroxides of Al and Fe. He also states that satisfactory results are obtainable if sufficient acid is added to dissolve the whole of the precipitated hydroxides and the excess of acid titrated back with alkali and methyl

red until the red colour changes to brownish yellow. Belgrave [1927] uses phenol red for titrating soil extracts containing $AlCl_3$. Rice-Williams [1929] used phenolphthalein and points out that any ferric and aluminium chloride in the solution will be estimated as free acid. He also recommends that the liquid should be boiled to expel CO_2 for special accuracy.

It may be stated at once that the lack of sharpness in the end point is not due to the presence of CO_2 but due to the incomplete hydrolysis of aluminium chloride. The observation of Rice-Williams that methyl orange is useless for the titration of soil extracts and the author's observation that a CO_2-free solution of $AlCl_3$ gives an indefinite end point when titrated in the cold with CO_2-free alkali using phenolphthalein as indicator, support the above view. If CO_2 were the cause of the disturbance the pink colour of the liquid with phenophthalein or the yellow colour with methyl red produced in the cold by adding alkali ought to persist even on boiling but the reverse was found to be the case.

It was therefore decided to study the titration of pure solutions of $AlCl_3$, $FeCl_3$, and $MnCl_2$ with alkali using various indicators.

If a solution of $AlCl_3$ is titrated for acidity and for chloride ion the two values should be identical. This identity was observed in the case of HCl when titrated with alkali using methyl red, phenol red, cresol red and phenolphthalein as indicators and with silver nitrate. On the other hand with pure $AlCl_3$ most of the indicators gave acidity figures lower than the chloride content. With phenolphthalein as indicator in boiling hot solution values agreeing with the chloride titration were obtained. The values using cresol red were only very slightly less. The titration data are shown in Table I.

TABLE I.

Titration of 25 c.c. of $AlCl_3$ with 0·1 N $AgNO_3$ and lime water.

Volume of $AgNO_3$ required . . . . . =5·5 c.c.
Calculated volume of lime water (0·03754 N) . . . =14·65 c.c.

No.	Indicator	pH at end point	Vol. of lime water reqd. c.c.	Remarks
1	Methyl red	5·1	13·3	Cold.
2	Do.	5·1	13·6	Boiled and cooled.
3	Do.	5·1	13·85	Boiled and titrated hot.
4	Phenol red	7·7	13·9	Cold.
5	Do.	7·7	14·25	Titrated hot.
6	Cresol red	8·2	14·5	Do.
7	Phenolphthalein	9·7	13·7	Cold.
8	Do.	9·7	14·65	Titrated hot.

Crookes states (Select Methods in Chemical Analysis: p. 159) that "it is an interesting fact that alumina is alkaline to methyl orange and acidic to litmus solution" and on this basis describes the titration of sodium aluminate, by Gatenby's process. According to this titration of a solution of the sample with *N* HCl and phenolphthalein gives the NaOH; further titration of the same solution in the cold with *N* HCl and methyl orange gives the alumina with sufficient accuracy. In the second stage of this titration aluminium hydroxide functions as an alkali. An attempt was therefore made to titrate $AlCl_3$ by adding an excess of lime water or NaOH and titrating back the alkali in presence of phenolphthalein in the cold. Near the end point the colour became very pale and the actual end (*i.e.*, the solution becoming colourless) was not sharp. With methyl red as indicator no definite end point could be obtained showing that $Al(OH)_3$ behaves like an alkali towards this indicator. It would appear therefore that when a solution containing alkali and $Al(OH)_3$ is titrated with acid some of the acid is taken up by the $Al(OH)_3$ to form $AlCl_3$ and thereby an overtitration with acid will be required to attain the end point. Titration in boiling solution, however, gave the correct end point. The results obtained by the alternative procedure are given in Table II.

TABLE II.

Titration of 25 c. c. of $AlCl_3$ after adding excess of alkali.

Volume of 0·1 $N AgNO_3$ required . . . . =8·1 c. c.

Calculated volume of lime water (0·0366 *N*) . . =22·1 c. c.

Phenolphthalein was used as indicator.

No.	Lime water added c. c.	Vol. of acid c.c. 0·0366*N*	Excess of acid reqd. over 22·1 c. c.	Remarks
1	22·1	..	..	Direct titration of hot solution.
2	30·0	8·05	0·15	Cold.
3	26·2	4·25	0·15	,,
4	26·5	4·55	0·15	,,

It was also found that in experiments 2, 3 and 4 the solutions required exactly 0·15 c. c. of lime water to restore the pink colour in boiling solution. However, the error involved in this method is negligible. Apart from the sharpness of the end point, the use of a stronger solution of acid appears to be liable to cause greater error as it would react with $Al(OH)_3$ more readily than the very dilute acid.

The titration of ferric chloride with alkali did not cause any difficulty. A short boiling near the end point was sufficient to obtain the correct value. The results are shown in Table III.

TABLE III.

Titration of 25 c. c. of $FeCl_3$ with $AgNO_3$ and lime water.

Volume of $AgNO_3$ required . . . . =3·9 c. c.

Calculated volume of lime water . . . =10·65 c. c.

No.	Indicator	Vol. of lime water	Remarks
1	Cresol red	10·65 c. c.	Boiled and titrated hot.
2	Penolphthalein	10·70 c. c.	Do.

Unlike ferric and aluminium chlorides a cold solution of manganese chloride was found to be neutral (or alkaline) to all the indicators tried. Even one drop of lime water added to a solution of $MnCl_2$ in presence of phenolphthalein caused a pink colour. Methyl red showed slight alkalinity directly. Phenol red showed an almost neutral reaction. When the solution of $MnCl_2$ was heated in presence of lime water acidity developed progressively towards phenolphthalein and cresol red but methyl red showed persistent alkalinity. The titration was therefore possible only with the first two indicators. During the titration a brown precipitate was formed, presumably hydrated manganic oxide. The absorption of oxygen and the consequent formation of the precipitate appeared to be essential to attain the end point. By boiling repeatedly between additions of lime water the end point was reached fairly rapidly. The pink colour of phenolphthalein persisted for at least five minutes (sometimes much longer) but afterwards the colour faded and disappeared probably due to the further oxidation of manganese which caused a further absorption of lime. The titration values are given in Table IV.

TABLE IV.

Titration of 25 c. c. of $MnCl_3$ with $AgNO_3$ and lime water

Volume of 0·1 *N* $AgNO_3$ required . . . . =6·7 c. c.

Calculated volume of lime water (0·03754 *N*) . . =17·8 c. c.

No.	Indicator	Lime water required c. c.	Remarks
1	Methyl red	*Nil*	Cold.
2	Phenol red	One drop	,,
3	,, ...	1·5 c. c.	Boiling hot—too slow.
4	Cresol red	17·4 c. c.	Boiling hot.
5	Phenolphthalein	17·8 c. c.	,,
6	,, ...	17·85 c. c.	,,

In order to determine the nature of the oxidation product the precipitate obtained in the phenolphthalein titration was treated with potassium iodide and HCl and the iodine liberated was titrated with standard thiosulphate. In two experiments 2·3 c.c. and 2·4 c.c. of decinormal thiosulphate were required. These correspond to the formation of Mn_3O_4, usually known as "foxy" or "red" batch sometimes formed in Weldon's process for the recovery of MnO_2 (Partington : The Alkali Industry : p. 116).

From these experiments it may be concluded that when soil extracts containing HCl and the chlorides of Fe, Al, Mn, Ca, Mg, K and Na are titrated in boiling solution with alkalies using phenolphthalein as indicator, the HCl and chlorides of Fe, Al and Mn will be accurately returned as acid. Any replaceable ammonium present as the chloride will also be included as acid. The titration values for soil extracts are shown in Table V.

TABLE V.

Titration of 25 c. c. of soil extract with lime water (0·03754N).

Indicator	Volume of lime water required in c. c.							
	9	9a	10	10a	11	11a	12	12a
Methyl red . . .	33·05	32·65	32·75	33·7	..	..	..	..
Phenol red . . .	34·1	33·9	34·0	34·7	..	..	..	..
Cresol red . . .	34·8	34·35	34·6	35·55	35·9	35·1	35·5	37·0
Phenolphthalein . .	34·9	34·4	34·65	35·65	35·9	35·2	35·5	37·1

These data show that the use of methyl red and phenol red is liable to cause distinctly high values for total bases.

Analysis of the soil extracts.

In order to have a check on the values for total bases obtained by titration the Ca, Mg, K and Na were estimated in each sample by direct methods and the totals obtained by addition. Although Mn was present in the extracts it was included as acid in the titration and therefore it was not estimated.

Calcium.—200 c.c. of the extract was evaporated to dryness, treated with 5 c. c. of conc. HNO_3 and again evaporated to dryness. The residue was gently ignited and extracted with nitric acid. The Fe, Al and Mn were removed by treatment with NH_4Cl, NH_4OH and 10 c. c. of hydrogen peroxide followed by boiling. Although the removal of Mn by H_2O_2 is liable to cause slight loss of Ca under strongly alkaline conditions, the method is very covenient and the loss is negligible, as the amount of Mn is extremely small. The calcium in the filtrate was precipitated with a slight excess of ammonium oxalate, and the calcium oxalate titrated with standard $KMnO_4$.

Magnesium.—The filtrate from the calcium estimation was evaporated to small volume and after acidifying the liquid with a little HCl the magnesium was precipitated from the acid medium as recommended by Miss Epperson [1928] by adding excess of a 10 per cent. solution of ammonium phosphate and neutralising with a slight excess of ammonia. After stirring for a few minutes to induce crystallisation, about 10-15 c. c. of strong ammonia was added and the liquid left covered overnight. The precipitate of magnesium ammonium phosphate was purified by reprecipitation under similar conditions as before. After standing four hours the precipitate was filtered and washed with dilute ammonia. The beaker and filter were exposed to the air for about 1 hour to remove the free ammonia and then the precipitate was dissolved in excess of standard acid and titrated with alkali and methyl orange as in the method of Handy [1900]. Close agreememt was obtained with the standard pyrophosphate method. The results are shown in Table VI.

Table VI.

Magnesium in soils. (Milli-equivalents per cent.)

Soil	Handy's method	Pyrophosphate method
1	22·4	21·76
1a	23·2	23·96
2	22·1	22·72
2a	21·9	22·28
Average	22·40	22·68

Potassium.—This was estimated by the cobaltinitrite method essentially as described by Dodd [1924] and Milne [1929]. 200 c. c. of the extract was evaporated to dryness with the addition of 10 c. c. of conc. HNO_3 and the residue gently ignited on a wire gauze for a few minutes, and when cold extracted with water and filtered. The filtrate was evaporated to a few drops, the reagents were then added and the whole evaporated to dryness on the water bath. When quite cold acetic acid was added and after standing half an hour sufficient water was added to dissolve the soluble salts. The precipitate was filtered through asbestos in a Gooch crucible, washed with dilute sodium sulphate and titrated with 0·05 *N* potassium permanganate and oxalic acid. The potash was calculated by the theoretical factor. The results agreed with those obtained by the standard perchlorate method and are shown in Table VII.

TABLE VII.

Potassium in soils. (*Milli-equivalents per cent.*)

Soil	Cobaltinitrite method	Perchlorate method
1	1·28	1·25
1a	1·18	1·16
2	1·18	1·16
2a	1·27	1·25
Average	1·25	1·21

Sodium.—This was estimated in 100 c.c. of the extract by the method of Barber and Kolthoff [1928]. The extract was evaporated to dryness with HNO_3, gently ignited on a wire gauze, extracted with water and filtered. The filtrate was evaporated to dryness and after cooling, the residue was treated with 15 c. c. of uranyl zinc acetate reagent as recommended by Blenkinsop [1930]. The precipitate was filtered off after two hours through a Gooch crucible and after washing with small portions of the reagent, alcohol and ether successively the crucible was allowed to dry in the air and then weighed. When the filtration was postponed for two days as in the procedure of Bray [1928] the evaporation of the liquid had caused the deposition of a large amount of solid which could not be removed by solution, and the experiments had to be repeated.

The values for total bases obtained by titration and by addition of the separate bases, and also the exchangeable bases after correction for carbonates are shown in Table VIII.

COMPARISON OF THE RESULTS WITH THOSE OBTAINED BY ELECTRODIALYSIS.

Previous experience with the electrodialysis method of Crowther and Basu [1929] had shown that the values obtained for total bases and Ca and Mg in acid soils are reproducible and in agreement with those obtained by standard methods. On the other hand with calcareous soils prolonged electrodialysis caused continued removal of bases. Although the Ca was not much affected the total bases and Mg had increased very considerably; Some of these observations are shown in Table IX. A comparison of the results obtained by the author's titration method with those obtained by electrodialysis with four acid soils showed satisfactory agreement, but a similar comparison was not attempted in the case of calcareous soils. The data for acid soils are given in Table X. It should be pointed out that in both methods the total bases were obtained by similar titration.

TABLE VIII.

Total and exchangeable bases in soils. (*Milli-equivalents per cent.*)

Soil	Ca	Mg	K	Na	Total bases addn.	Total bases titration	Carbonate	Exchangeable bases
21	30·60	10·42	1·18	1·18	43·38	43·12	9·10	34·02
21a	27·36	11·04	1·13	1·56	41·09	40·72	7·70	33·02
22	31·32	7·67	1·16	1·47	41·62	41·76	10·00	31·76
22a	27·20	10·26	1·00	2·14	40·60	40·72	10·00	30·72
23	29·88	9·00	1·04	1·11	41·03	40·96	9·10	31·86
23a	26·64	10·23	1·06	1·39	39·32	39·60	8·20	31·40
24	26·64	9·30	1·08	1·51	38·53	38·80	7·70	31·70
24a	26·64	10·02	0·98	1·92	39·56	39·76	8·20	31·56
25	35·28	8·00	0·96	1·54	45·78	46·08	13·20	32·88
25a	32·40	8·00	0·84	2·31	43·55	44·16	13·20	30·96
26	29·88	6·00	0·89	1·23	38·00	38·88	9·55	29·33
26a	27·72	11·40	0·84	1·86	41·82	41·60	10·45	31·15
27	31·68	7·60	0·87	1·45	41·60	42·24	10·45	31·79
27a	32·40	8·20	0·82	2·10	43·52	43·84	8·20	35·64
28	33·12	10·00	0·85	1·40	45·37	44·40	10·45	33·95
29	31·00	10·00	1·34	1·85	44·19	44·00	10·45	33·55
29a	31·00	13·00	1·20	2·37	47·57	47·20	9·60	37·60
30	28·20	11·20	1·42	1·66	42·48	41·92	9·10	32·82
30a	27·70	12·20	1·24	2·41	43·53	43·20	8·20	35·00
F18	9·30	6·50	0·53	0·56	16·89	16·72	..	16·72
F18a	9·68	6·44	0·35	0·48	16·96	17·12	..	17·12
F28	15·24	5·92	0·46	0·31	21·93	22·16	..	22·16
F28a	15·68	5·92	0·34	0·28	22·22	22·16	..	22·16
K1	270·00	0·60	2·05	22·95	295·60	296·00	253·60	42·40
K2	115·80	6·20	2·15	22·78	146·93	147·20	104·50	42·70
K3	684·00	281·40	1·23	6·21	972·84	973·20	955·00	18·20
K4	334·80	88·80	1·60	13·02	438·22	438·40	422·70	15·70
K5	508·00	161·20	1·00	4·83	675·03	675·20	644·10	31·10
K6	494·00	*Nil.*	0·96	12·00	506·96	507·20	409·90	97·30
M1	0·85	0·72	0·22	0·21	2·00	2·04	..	2·04
M2	3·50	2·05	0·38	0·29	6·22	6·25	..	6·25

TABLE IX.

Exchangeable bases in soils by electrodialysis.

Soil	pH	Time	Total bases m.e.	Ca m. e.	Mg m.e.	Remarks
147a . . .	5·6	9 hours	5·1	—	—	b, c, d are duplicates.
147b . . .	...	10½ ,,	5·4	4·53	—	
147c . . .	...	12½ ,,	5·6	4·57	—	
147d . . .	...	14½ ,,	5·5	—	—	
81a . . .	5·8	23 ,,	14·25	—	—	
81b . . .	...	23 ,,	14·10	—	—	
81c . . .	...	14½ ,,	14·0	—	—	
Mandalay a . .	8·05	18 ,,	40·3	31·5	6·7	Calcareous.
,, b . .	...	73 ,,	41·0	—	—	
,, c . .	...	11 days	46·5	32·7	10·6	
,, d . .	...	11 ,,	47·25	—	—	

TABLE X.

Exchangeable bases in acid soils. (Milli-equivalents. per cent.)

Soil	Author's method	Electrodialysis method
F. 18	16·72	16·90
F. 18a	17·12	17·20
F. 28	22·16	21·40
F. 28a	22·16	22·90
Average .	19·65	19·60

DISCUSSION.

The use of 0·05 *N* HCl for base exchange work has been objected to as it is supposed to decompose the exchange complex. The extraction of Fe and Al by

this reagent has been attributed to this decomposing action. Novac and Malac [1927] have compared the exchangeable bases extracted from a podsol soil by dil. HCl with those extracted by NaCl, NH_4Cl and $BaCl_2$. The data show that the total exchangeable bases as well as the separate bases Ca, Mg and K extracted by all the reagents are in close agreement. Mattson [1926] has similarly compared the values of the exchangeable bases in two soil colloids by extraction with *N* NH_4Cl, 0·05 *N*HCl and by electrodialysis. The agreement between the three methods is very close for total and individual bases. The extraction of silica and sesquioxides is greatest by HCl, somewhat less by electrodialysis and least by NH_4Cl.

In an important research on the nature of the base exchange complex in soils Kerr [1928] prepared the acid corresponding to the complex without causing decomposition by extracting the replaceable bases with 0·1 *N* HCl. He also found that *N* HCl decomposed the complex and utilised this fact for its analysis.

The ability to dissolve sesquioxides is apparently not confined to HCl only. Novac and Malac have given data for an acid podsol soil No. 107 which show that the amount of sesquioxides dissolved by 0·05 *N* HCl from all the horizons (60-70 m. e. in A, and A2 ; and 5-10 m. e. in B and C) is almost the same as that extracted by normal solutions of NaCl, NH_4Cl, or $BaCl_2$. Further, McGeorge [1929] has shown that during electrodialysis of Hawaiian soils much iron, aluminium and manganese passed into the dialysate and that the residual soils from this treatment had almost the same exchange capacity as the original soils. It is therefore evident that the oxides of Fe, Al, and Mn arise from sources other than the exchange complex. Parker [1929] has shown that Fe and Al are not present in exchangeable forms in the soil. Although Mn has been proved to be exchangeable by Schollenburger [1927] the amount of Mn normally present in this form is quite small.

The existence of alumina and ferric oxide in the soil in the free state in hydrated and colloidal forms is well known and recently Hardy [1931] has described a method for their estimation. It is to be expected that such reactive forms of the sesquioxides will be dissolved by HCl. Neutral salts are also capable of dissolving them by indirect action from acid soils. For instance NH_4Cl liberates considerable acidity by exchange reaction with the hydrogen ions of the complex ; ammonium acetate dissolves aluminium hydroxide from the soil by peptising action as stated by Schollenburger and Dreibelbis [1930]. In acid organic soils, such as the podsols, Fe and Al, may be present as humates which are decomposable by acids and neutral salts. There is also the possibility that the Fe and Al, of organic soils exist as colloidal hydroxides protected by the humus. In the latter case the dissolving action of neutral salts may be due to the acidity developed by inter-

action of the humus with the salts. An examination of the condition of Fe and Al in organic soils by Hardy's method will therefore be of interest.

It may be concluded that bases are liable to be present in soils as carbonates, silicates, humates and hydroxides in addition to the amount in exchangeable form and that whatever reagents are employed for the extraction of exchangeable bases some portion at least of the extraneous material will be dissolved. A correction is easily applied for the carbonates dissolved by determining CO_2. This seems to be the only correction that has been applied till now. According to Thomas [1929] it seems to be feasible also to apply a correction for the silicates decomposed by estimating the silica liberated and assuming this to be present as metasilicate. The humates are to be regarded as exchangeable but only a small amount of work has been done on this subject. If hydroxides such as magnesium hydroxide are present in soils no correction is possible for these. In any case when a soil contains Ca and Mg in exchangeable forms and also as carbonates and silicates there is no method for determining the separate amounts. Although the method described in this paper was worked out originally for slightly calcareous soils, numerous experiments have shown that it can be used equally well for highly calcareous soils and also for soils poor in bases. So far the author had no opportunity to examine the highly leached organic soils of Assam and Malaya which are reported to yield coloured extracts but it is hoped to deal with them in the near future.

Summary.

(1) A simple method has been described for determining the total exchangeable bases in soils. The soil is leached with approximately 0·05 *N* HCl and the leachate titrated for acidity and total chloride. The difference between these titrations corresponds to the chlorides of Ca, Mg, K and Na. The bases present as carbonates are corrected for by a CO_2 determination.

(2) Various indicators suitable for the titration have been tested and the conditions for the accurate alkalimetric titration of the chlorides of Fe, Al, and Mn determined.

(3) Data are presented to show the nature of the agreement obtained by different methods for the estimation of magnesium, potassium and total bases.

In conclusion, the author wishes to express his gratitude to Mr. J. Charlton, M.Sc., F.I.C., Agricultural Chemist, Burma, for encouragement and advice, and for permission to publish this paper.

REFERENCES.

Barber, H. H. and Kolthoff, I. M. (1928). *J. Amer. Chem. Soc.* **50**, 1625-31.
Basu, J. K. (1931). *J. Agric. Sci.* **31**, 487.
Belgrave, W. N. C. (1927). *Malayan. Agric. J.* **17**, 206-209.

Blenkinsop, A. (1930). *J. Agric. Sci.* **20**, 511-516.
Bradfield, R. (1927). Proc. First Int. Cong. Soil Sci. Comm. II, Vol. **2**, pp. 264 278.
Bray, R. H. (1928). *J. Amer. Soc. Agron.* **20**, 1160-1166.
Bray, R. H. and Willhite, F. M. (1929). Ind. Eng. Chem. (Anal. Ed.), Vol. 1, p. 144.
Burgess, P. S. (1929). *J. Amer. Soc. Agron.* **21**, 1040-1044.
Crowther, E. M. and Basu, J. K. (1929). Trans. Sec. Comm. Int. Soc. Soil Sci. (Budapest), Vol. A, pp. 100-102.
Dodd, A. H. (1924). *J. Agric. Sci.* **14**, 148.
Epperson A. W. (1928). *J. Amer. Chem. Soc.* **49**, 321.
Gedroiz, K. K. (1918). *J. Exp. Agron.* (Russian), **19**, 226 (seen in abstract).
Handy, J. O. (1900). *J. Amer. Chem. Soc.* **22**, 31-39. See also W. W. Scott. (Standard Methods of Chemical Analysis. Vol. 1, p. 294.)
Hardy, F. (1931). *J. Agric. Sci.* **21**, 150-166.
Hissink, D. J. (1923). *Soil Sci.* **15**, 269-276.
——— (1925). Tran. Forad. Soc. Vol. 20, pp. 560-566.
Humfeld, H. (1928). *J. Amer. Soc. Agron.* **20**, 1146.
Humfeld, H. and Alben, A. O. (1927). *J. Amer. Soc. Agron.* **19**, 984-993.
Joseph, A. F. and Oakley, H. B. (1929). *J. Agric. Sci.* **19**, 121.
Kappen, H. (1929). Br. Chem. Abs. (B), p. 487.
Kelley, W. P. (1917). *J. Amer. Soc. Agron.* **9**, 285-295.
——— (1929). *J. Amer. Soc. Agron.* **21**, 1028.
Kelley, W. P. and Brown, S. M. (1924). Calif. Agri. Exp. Sta. Tech. paper 15.
Kerr, H. W. (1928). *Soil Sci.* **26**, 385.
Mattson, S. (1926). *J. Agric. Res.* **33**, 563-567.
——— (1927). Proc. First Int. Cong. Soil Sci. Comm. II, Vol. 2, pp. 199-211.
McGeorge, W. T. (1929). The Hawaiian Planter's Record, Vol. 33, pp. 346-364.
McIntire, W. H. and Shaw, W. M. (1930, 1). *J. Amer. Soc. Agron.* **22**, 14-27.
——— (1930, 2). *J. Amer. Soc. Agron.* **22**, 919-934.
Menchikowsky, F. and Ravikovitch, S. (1929). *Soil Sci.* **27**, 49.
Milne, G. (1929). *J. Agric. Sci.* **19**, 541.
Novac, V. and Malac, B. (1927). Bul. Czechoslovak. Acad. Sci. Vol. 3, pp. 588-594 (seen in abstract).
Parker, F. W. (1929). *J. Amer. Soc. Agron.* **21**, 1034.
Puri, A. N. (1930). *Agric. Res. Inst. Pusa Bull.*, 206.
——— (1931). *Soil Sci.* **31**, 275-279.
Ravikovitch, S. (1930). *Soil Sci.* **30**, 82.
Rice-Williams (1929). *J. Agric. Sci.* **20**, 589.
Schollenburger, C. J. (1927). *Science* **65**, (N. S.), 552-553.
Schollenburger, C. J. and Dreibelbis, F. R. (1930, 1). *Soil Sci.* **29**, 387.
——— (1930, 2). *Soil Sci.* **30**, 161-173.
Spurway, C. H. (1925). *Soil Sci.* **19**, 399.
Thomas, M. D. (1928). *Soil Sci.* **25**, 390.
Turner, P. E. (1928). *J. Agric. Sci.* **18**, 257-265.
Tyurin, I. W. (1927). Le Pedologie (Russian) No. 1, pp. 5-24 (seen in abstract).
Wilson, B. D. (1928). *Soil Sci.* **26**, 407-417.
——— (1929). *Soil Sci.* **28**, 411-421.
——— (1930). *Soil Sci.* **29**, 94.

FOOD PLANTS OF *DIALEURODES CITRI*, ASHMEAD. (ALEYRODIDAE). IS JASMINUM ONE OF THEM?

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I. INTRODUCTION.

It cannot be sufficiently emphasized that for a proper control of any insect pest, full and accurate information regarding its food plants is absolutely indispensable. The eradication of the alternative hosts as an effective method of insect control

has wide application and the elimination of the centres of infection is always an important consideration. For the control of *Dialeurodes citri**, Ashm., Berger [1908, 1910] recommended that the alternative hosts 'should be cut down and immediately burned' and he did not spare even the ornamental plants. "It seems akin to vandalism," says Berger, "to sacrifice some of the ornamentals, but all successful warfare consists, in some degree at least, in reducing the number of the enemies' strongholds." Such an action can only be taken after a very thorough inquiry, because if the sweet scented jasminum had been destroyed in India to safeguard the citrus trees from the attack of *D. citri*, the verdict of 'vandalism' would have become appropriate.

Unfortunately, not uncommonly, sufficient care is not exercised in determining the alternative hosts of even some of our commonest pests. The possibility of the same insect having different food plants in different countries is admitted, and a change in diet is also not an uncommon phenomenon among insect pests. In such cases, however, it is still more essential to obtain an accurate determination of the pest concerned and authentic records of its food plants.

In the United States of America investigations on *D. citri*, Ashm. were carried out mainly during 1903-10. At that time very little taxonomic work had been done on the Aleyrodidæ, and Quaintance and Baker were just laying the foundation of their excellent studies of this family. The difficulties of accurate determination of the Aleyrodidæ have been sufficiently impressed by Morrill and Back [1911].

Gossard [1903], Berger [1908, 1910], Morrill and Back [1911], and Woglum [1913] made observations on the food plants of *D. citri* in the U. S. A. and Woglum visited the Orient including India. Fletcher [1914, 1920], Misra [1923] and Lamba [1931] have recorded the food plants of this pest from various parts of India, particularly Pusa. The present authors have studied the problem in the Punjab, and have examined material obtained from Pusa and Nagpur.

II. Alternative food plants.

Below is given a full list of the plants so far mentioned by different investigators as the alternative hosts of *D. citri*. As will appear from what follows, the authenticity of this record is questionable in some cases, and in a few others the information is certainly incorrect.

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* Synonymy :—

Aleyrodes citri, Ashmead. Fl. Dispatch, n. sr. Vol. II, 1885.

Aleyrodes citri, Riley & Howard. Insect Life, V. no. 4, p. 219, 1893.

Aleyrodes eugeniæ var. *aurantii*, Mask. Trans. & Proc. N. Zeal. Ins. V. 28, p. 431, 1895.

Aleyrodes aurantii, Ckll. Fl. Agric. Exp. Sta. Bull. 67, 665, 1903.

Dialeurodes citri (Ashmead.), Quaintance & Baker. J. A. R. Vol. VI, 1916, p. 469.

The list of the food plants (authentic and questionable) of D. citri, *other than Citrus spp. (Rutaceæ).*

No.	Food plants	Family	Status	Country	References (see p. 253)
1	*Sabal megacarpa* (Scrub Palmetto)	Palmæ	* O. P.	Florida	1, 2.
2	*Smilax* sp.	Liliaceæ	,,	,,	1, 2, 8.
3	*Quercus aquatica*	Cupuliferæ	‡ ?	,,	5.
4	*Quercus nigra* (Water oak)	,,	O. P.	,,	2.
5	*Ficus altissima*	Urticaceæ	,,	,,	1, 2, 5.
6	*Ficus* sp.	,,	,,	Costa Rica	1, 2, 5.
7	*Ficus macrophylla*	,,	?	Florida	8.
8	*Magnolia fuscatum* (Banana shrub)	Magnoliaceæ	O. P.	,,	8.
9	*Michelia fuscata* (Banana shrub)	,,	,,	,,	1, 2.
10	*Laurus nobilis*	Lauraceæ	,,	California	8.
11	*Prunus carolinum*	Rosaceæ	,,	Florida	5.
12	*Pyrus* spp.	,,	,,	,,	8.
13	*Prunus laurocerasus* (Cherry laurel).	,,	,,	,,	8.
14	*Rubus* sp. (blackberry)	,,	,,	,,	2.
15	*Laurocerasus caroliniana*	,,	,,	,,	1, 2.
16	*Xanthoxylum* sp.	Rutaceæ	,,	,,	5.
17	*Xanthoxylum clavaherculis*	,,	† P.	,,	8.
18	*Fagara clava Herculis* (Prickly ash).	,,	,,	,,	1, 2.
19	*Ailanthus glandulosa*	Simarubiaceæ	..	California	7.
20	*Melia azedarach*	Meliaceæ	P.	Florida	1, 2, 5, 8.
21	*Melia azedarach umbraculifera*	,,	,,	,,	1, 2, 8.
22	*Hiptage mandalobata*	Malpighiaceæ	,,	India	13.

* O. P.—Occasionally preferred.
† P.—Preferred.
‡ ?.—The authority for these food plants is not known.

No.	Food plants	Family	Status	Country	References (see p. 253)
23	*Camellia japonica*	Ternstrœmiaceæ.	O. P.	Florida	1, 2.
24	*Punica granatum* (Pomegranate)	Lythraceæ	,,	,,	8.
25	*Myrtus lagerstrœmia*	Myrtaceæ	..	California	7.
26	*Eugenia jamboos*	,,	..	India	6.
27	*Hedera helix* (Eng. ivy)	Arabiaceæ	..	California	7.
28	*Ardesia humulis*	Myrsinaceæ	..	India	6.
29	*Diospyros kaki*	Ebenaceæ	P.	Florida	1, 2, 5.
30	*Diospyros virginiana*	,,	,,	,,	1, 2, 8.
31	*Ligustrum amurence*	Oleaceæ	,,	,,	5.
32	*Ligustrum* spp.	,,	,,	,,	5, 8.
33	*Syringa* sp. (Lilac)	,,	,,	,,	8.
34	*Osmanthus americanus* (Wild olive or devil-wood).	,,	O. P.	,,	2, 8.
35	*Fraxinus lanceolata* (green ash)	,,	,,	,,	2, 8.
36	*Jasminum sambac*	,,	P.	India	4, 6, 7.
37	*Jasminum arborescens*	,,	,,	,,	4, 6, 7.
38	*Jasminum ororatissimum*	,,	?	California	8.
39	*Nerium oleander*	Apocynaceæ	O. P.	Florida	1, 2.
40	*Allamanda neriifolia*	,,	,,	,,	1, 2, 8.
41	*Gardenia jasminoides*	Rubiaceæ	P.	,,	1, 2.
42	*Gardenia florida* (Cape Jasmine)	,,	,,	,,	5.
43	*Cephalanthus occidentalis* (Button bush).	,,	O. P.	,,	2.
44	*Coffea arabica*	,,	,,	,,	1, 2, 8.
45	*Viburnum nudum*	Caprifoliaceæ	,,	,,	1, 2, 5, 8.
46	*Lonicera japonica* (Honey suckle)	,,	,,	,,	2.

A great deal of uncertainty has always existed regarding the food plants of *D. citri*. Berger [1908] has expressed his doubts regarding the water oak and scrub palmetto, and with regard to *Rubus* sp., *Quercus nigra*, *Sabal megacarpa*, *Lonicera japonica*, *Ficus altissima* and *Ficus* sp. (from Costa Rica) he is not sure as to 'whether *A. nubifera** or *A. citri* or both infest them', although he is inclined to believe that it is the latter.

In some cases a plant observed to have an occasional egg and first stage larva has been elevated to the dignity of a food plant, as has been done by Berger [1908] in the case of *Camellia japonica*.

Morrill and Back [1911] confined Citrus White Fly adults on *Quercus brevifolia*, *Magnolia fœtida*, *Rubus* sp., *Prunus caroliniana* (Laural cherry or Mock olive), *Ficus carica* (cultivated figs), and *Myrtus lagerstrœmia* (Crape myrtle) and observed that all the adults had died within four days without ovipositing, while the check lots deposited eggs on the citrus leaves in a normal manner.

In U. S. A. the China and Umbrella trees (*Melia* sp. and var.) are regarded as the most preferred of the food plants [Morrill and Back, 1911]. By some, these trees are regarded as the original host plants of *D. citri*. In India, Woglum [1913] did not find a single case of a China tree being infested with this pest, 'although in some cases the foliage of this tree came in contact with aleyrodidæ-infested orange plants.' Our observations confirm Woglum's conclusions. In fact even under experimental conditions we were not able to transfer *D. citri* to *Melia azedarach*.

III. Jasminum, the alleged favoured food plant or original host of *D. citri*.

Woglum came out to India in 1910 in search of the native home of *D. citri*, and to discover its enemies. He visited all the important citrus growing parts of the country, from Peshawar in the extreme North-West to the Khasia hills of Assam in the East, from Sikkim below Tibet to Nagpur in the Central Provinces and Poona in Bombay. Everywhere in this territory, where citrus grew, he found *D. citri*. Besides *Citrus* spp. he mentions *Jasminum sambac* and *Hiptage mandalobata* as the food plants of this pest. And regarding the former he says:

> "In India the White Fly prefers jasminum as a host plant over citrus trees. On this plant the insect was of much greater occurrence and capable of withstanding climatic conditions better than on any other host. Viewing the problem entirely from the stand-point as seen by the writer in India it would appear that jasminum was the original host rather than citrus."

* Syn. *Dialeurodes citrifolii* (Morgan).

Fletcher [1920] records *Jasminum sambac* as the food plant from Pusa. Misra [1923] records *Jasminum sambac* and *J. arborescens* as the alternative and favoured food plants of *D. citri*. Lamba [1931] also includes jasminum among the food plants of *D. citri*.

IV. Field observations in the Punjab.

During the course of our investigations on the Aleyrodidæ of citrus in the Punjab, we have had numerous opportunities of carefully examining all types of vegetation, cultivated and wild, growing in and around citrus orchards, but so far *D. citri* has not been found on any plant other than *Citrus* spp. The plants previously reported as the alternative hosts such as *Jasminum* spp., *Melia azedarach*, *Punica granatum* (pomegranate), *Ficus religiosa*, *Pyrus communis* (pears), were carefully examined in Dera Ghazi Khan, Muzaffargarh, Multan, Lyallpur, Sargodha, Gujranwala, Gujrat, Lahore, Amritsar, Gurdaspur, Jullundur, Ferozepore, Ambala and Gurgaon, but in no case was *D. citri* found on any of them. Of course various other species of Aleyrodidæ were collected from all these plants with the exception of *Melia azedarach*, from which so far we have not obtained any species of the White Flies [confirming Woglum, 1913].

It will not be out of place to mention that Woglum [1913] carried out his observations in the Botanical garden, Lahore, where he found citrus hedges severely attacked by *D. citri*. In this garden the commoner trees of almost all the families are growing in close proximity. The following plants in this garden were thoroughly examined in December 1931, but no evidence of *D. citri* infestation was discovered, while at the time of examination citrus hedges were badly infested:—

Smilax sanctifolia (Liliaceæ), *Quercus* sp. (Cupuliferæ), *Ficus religiosa*, *F. carica*, *F. ratanus* (Urticaceæ), *Magnolia grandiflorum* (Magnoliaceæ), *Pyrus communis*, *Prunus persica*, *P. domestica* (Rosaceæ), *Xanthoxylum album* (Rutaceæ), *Melia azedarach* (Meliaceæ), *Hiptage mandalobata* (Malpighiaceæ), *Eugenia jamboos*, *Eugenia jambolana* (Myrtaceæ), *Punica granatum* (Lythraceæ), *Diospyros kaki*, *D. cordifolia* (Ebenaceæ), *Jasminum sambac*, *J. humile*, *J. chrysantha*, *J. officinale*, *J. auriculatus*, *Jasminum* sp., *J. pubescense*, *J. grandiflorum*, *Ligustrum lucidum* (Oleaceæ), *Nerium odorum*, *N. album* (Apocynaceæ), *Ardesia humulis*, *A. pickeringa*, *A. solanaceæ* (Myrcinaceæ).

Practically everywhere in the Punjab, *Jasminum* spp. have been found highly infested with *D. kirkaldyi*, Kot. Plots of *Jasminum* growing close to orange trees were examined specially carefully. At Lyallpur in a big garden* a *Jasminum* plot

* S. Jiwan Singh, Chack No. 213, R. B. Lyallpur.

of about 4 *marlas* (1,089 sq. ft.) is located quite near the orange plantation. The *Citrus* are very badly infested with *D. citri* every year, while the *Jasminum* plants are attacked by *D. kirkaldyi*, but not by *D. citri*. Similar instances have been observed at Gujranwala, Sargodha and Lahore.

Woglum [1913] mentioned *Hiptage mandalobata* as one of the alternative food plants of *D. citri* in India. A vine growing in the Botanical Garden of the Punjab Agricultural College, Lyallpur, was examined and found to be lightly infested with *D. kirkaldyi*. A plot of *Jasminum* spp. and some citrus plants were also present nearby. *Jasminum* plants were attacked by *D. kirkaldyi* and citrus by *D. citri*, but in spite of the fact that both the species of the genus *Dialeurodes* were present, *Hiptage* was only attacked by *D. kirkaldyi*. Leaves of *Hiptage mandalobata* collected from the Botanical Garden, Lahore, also showed a very heavy attack of *D. kirkaldyi*, but were not infested with *D. citri*.

V. Breeding experiments.

The following experiments were conducted at the Entomological Field Laboratory, Lyallpur, for verification of the food plants of *Dialeurodes citri*, Ashm.

1. Adults of *D. citri* obtained from *Citrus* spp. were liberated under voile cloth sleeves on *Jasminum sambac*, possessing fresh growths of leaves. The experiments were repeated several times, but it was observed that adults died within 1-2 days without ovipositing. Only in one instance a few eggs were laid, but the nymphs died on hatching.

2. Similarly, a very large number of adults of *D. citri* were liberated on the following plants, but in no case did they oviposit: *Punica granatum* (Pomegranate), *Psidium guava* (Guava), *Melia azedarach* (China tree), *Ficus religiosa* (Pipal), *Ficus carica* (Fig), *Pyrus communis* (Pears), *Aegle marmelos* (Beal), and *Murraya exotica*.

3. Side by side control experiments were also conducted, a large number of adults of *D. citri* being liberated on citrus plants under similar voile cloth sleeves. In all cases, heavy oviposition was observed. The eggs hatched out and the nymphs successfully reached the adult stage.

4. The adults of *D. kirkaldyi*, Kot. from *Jasminum sambac* were sleeved on citrus plants but no oviposition was obtained.

5. Adults of *D. kirkaldyi*, Kot. from *Jasminum sambac* were also liberated on *Jasminum* plants and they were observed to breed successfully.

The following statement gives details regarding these experiments.

Experimental Record. (Locality: Lyallpur).

Date of sleeving	No. of adults sleeved	Plants on which sleeved	Life of adults on the plants	Date of oviposition	Date of hatching	Date of emergence	Remarks
Adults sleeved: *D. citri.*							
Plants from which taken: *Citrus.*							
			days				
7th September 1929.	315	*Jasminum sambac.*	1—2	..	..	..	No oviposition.
7th September 1929.	250	*Citrus medica*, var. *acita.*	6—7	8th to 15th Sept. 1929.	18th to 25th Sept. 1929.	25th Mar. 1930	Heavy oviposition.
7th September 1929.	245	*J. sambac* .	1—2	..	..	..	No oviposition.
7th September 1929.	340	*C. medica*, var. *acita.*	6—7	8th to 14th Sept.	19th to 27th Sept. 1929.	10th Mar. 1930	Heavy oviposition.
7th September 1929.	450	*J. sambac* .	1—2	..	..	..	No oviposition.
7th September 1929.	215	*C. medica*, var. *acita.*	6—7	8th to 12th Sept. 1929.	20th to 26th Sept. 1929.	10th Mar. 1930	Heavy oviposition.
15th March 1930.	335	*J. sambac* .	1—2	..	..	..	No oviposition.
15th March 1930.	340	*C. medica*, var. *acita.*	6—7	15th to 22nd Mar. 1930.	26th Mar. to 6th Apl. 1930	20th Aug. 1930	Heavy oviposition.
17th March 1930.	310	*J. sambac* .	1—2	18th Mar. 1930.	..	..	Only 8 eggs were laid but the nymphs died on hatching.
19th March 1930.	330	*C. medica*, var. *acita.*	6—7	19th to 26th Mar. 1930.	29th Mar. to 8th Apl. 1930.	20th Aug. 1930	Heavy oviposition.
25th August 1930.	61, 65, 52 & 85.	*Punica granatum*	1—2	..	..	..	4 replicates but no oviposition.
25th August 1930.	115, 75 & 85.	*Psidium guava* .	1—2	..	..	..	3 replicates but no oviposition.
25th August 1930.	80, 65, 45, 60.	*Melia azedarach*	1—2	..	..	..	4 replicates but no oviposition.
25th August 1930.	92, 85, 75.	*Ficus religiosa* .	1—2	..	..	..	3 replicates but no oviposition.
25th August 1930.	115, 66, 64.	*Pyrus communis*	1—2	..	..	..	3 replicates but no oviposition.
25th August 1930.	80, 95, 90.	*Aegle marmelos* .	1—2	..	..	..	3 replicates but no oviposition.
25th August 1930.	85, 90, 93.	*Murraya exotica*	1—2	..	..	..	3 replicates but no oviposition.
25th August 1930.	65	*C. medica*, var. *acita.*	1—2	27th Aug. 1930.	6th Sept. 1930	10th Apl. 1931	Heavy oviposition.
25th August 1930.	95	*C. medica*, var. *acita.*	6—7	27th to 29th Aug. 1930.	6th to 15th Sept. 1930.	17th Apl. 1931	Heavy oviposition.
Adualts sleeved: *D. kirkaldyi*, Kot.							
Plants from which taken: *Jasminum.*							
19th March 1930.	455	*C. medica*, var. *acita.*	1—2	..	..	..	No oviposition.
19th March 1930.	440	*Jasminum sambac.*	8—10	13th to 20th Mar. 1930.	25th to 31st Mar. 1930.	15th Aug. 1930	Heavy oviposition.
19th March 1930.	396	*C. medica*, var. *acita.*	1—2	..	..	..	No oviposition.
19th March 1930.	388	*Jasminum sambac*	8—10	19th to 27th Mar. 1930.	28th Mar. to 9th Apl. 1930.	15th Aug. 1930	Heavy oviposition.

VI. Examination of material from Pusa and Nagpur.

From the above it will appear, that *Jasminum* spp. are not the host plants of *D. citri* and that the species attacking these plants is *D. kirkaldyi*.

Through the courtesy of Mr. T. Bainbrigge Fletcher, Imperial Entomologist, *Jasminum* leaves infested with White Fly pupae and euparal mounts of pupae, were obtained from the Imperial Research Institute, Pusa. The slides were labelled: "*Dialeurodes citri* on *Jasminum*". On examination, however, the specimens were found to be *D. kirkaldyi*, Kot. The specimens on *Jasminum* leaves also belonged to the same species.

Specimens of *Jasminum* leaves attacked by White Fly were also obtained from Nagpur (Central Provinces) through the courtesy of Mr. K. R. Sontakay, Demonstrator, Agricultural College, Nagpur. In this case also, the specimens were found to belong to *D. kirkaldyi*.

VII. Woglum's facts and inferences.

Woglum's facts also support the conclusion arrived at. Woglum [1913] maintained that *Jasminum sambac* was the original host of *D. citri*. He says:—

> "The special point of interest is that these bushes are invariably infested with the white fly, and usually more or less severely. The writer has seen patches of this plant in which almost every leaf of each plant contained some living white flies. Bushes were sometimes found to be very black with sooty mould, a condition never seen in citrus trees. Not infrequently the writer has examined as many as a score of orange trees with the result of finding living material on only one or two, whereas every jasminum bush in the immediate vicinity would contain much living material".

He continues:

> ".....in all localities in which jasminum bushes were examined, which included Northern and Central India, these were found infested with *Aleyrodes citri*, and frequently somewhat severely. The white fly was found on citrus trees throughout this region, with the exception of Central Provinces and the Bombay Presidency; in these places it was seen only on jasminum....."

These facts simply show that *D. kirkaldyi* infests jasminum far more seriously than *D. citri* infests citrus and that *D. kirkaldyi* is far more widely spread than *D. citri*, which was not found by Woglum in the Central Provices and in Bombay.

Again Woglum states:—

> "In the Central Provinces no white flies were seen on citrus trees, yet in numerous instances jasminum bushes planted between the trees in

some cases even touching their trunks, contained many active insects."

This again shows that the species infesting jasminum does not attack citrus and that is what we have proved by actual experiments.

Lastly, Woglum puts forth the following as the final proof of his assertion that *Jasminum sambac* is the favoured food plant of *D. citri* : –

"Enough has already been stated to show the preference, in many instances in India, of the white fly for the jasminum rather than for citrus plants. The following additional evidence is itself conclusive : During May, while adult flies were emerging in large numbers on a jasminum bush, a number of small seedling orange trees of very tender foliage were placed immediately about the plant, so that the leaves of the orange trees, were in contact with those of the jasminum. Very few flies settled on those orange trees, while large numbers would be present on leaves of the jasminum within a few inches of the former. Even if the bush was so disturbed that the flies in their flight would settle on the orange trees they would ultimately desert these in order to go back to the original food plant."

This very experiment proves, more strongly than any thing else, that Woglum was dealing with two different species. The jasminum white fly *D. kirkaldyi*, Kot. naturally would not feed on citrus.

VIII. Distinguishing features of *Dialeurodes citri* Ashm. and *Dialeurodes kirkaldyi** Kot.

The pupae *D. kirkaldyi* may be distinguished from those of D. *citri* by their smaller size and by their general shape, more particularly by a distinct narrowing just behind the thoracic breathing folds and a brown median band on the thorax, which in the case of the pupae advanced in age, extends up to the vasiform orifice. The shape and number of teeth on the caudal margin of the vasiform orifice is also different in the two species. The adults can be easily distinguished by their

**Dialeurodes kirkaldyi*, Kot., was originally described by Kotinsky under the name of *Aleyrodes kirkaldyi*, Kot., in 1907. It was again described by Quaintance and Baker in 1917 and was named *Dialeurodes kirkaldyi*. Kot. The distinguishing features of the pupae of *D. citri* and *D. kirkaldyi* given by us agree with those given by Quaintance and Baker, but the observations about the fore-wing veins are our own. Regarding the food plants of *D. kirkaldyi* the only host recorded by these authors is *Jasminum*, but we have discovered another host, *viz.*, *Hiptage mandalobata*.

wings, in *D. citri* the radial sector has a sharp bend while in *D. kirkaldyi* it has only a slight curve. The distinguishing features are tabulated below :

Dialeurodes citri, Ashm. Female pupa.	*Dialeurodes kirkaldyi*, Kot. Female pupa.
1. Size: 1·4—1·5 × 1·08—1·2 m. m. *	*1. Size*: 1·2—1·28 × 1·0—1·1 m. m.
2. Shape: Subelliptical or broadly oval	*2 Shape*: Broadly oval but distinctly narrowed behind the thoracic breathing folds.
3. Colour: On the leaf, pale yellow with an orange or pale yellowish area in the middle. Under the microscope semi-transparent with a yellowish orange area.	*3. Colour*: Pale yellow with a median elongated dark brown band on the thorax, in some cases extending up to the vasiform orifice.
4. Vasiform orifice: Teeth on the caudal margin short, irregular in shape and a blunt process in the middle is evident.	*4. Vasiform orifice*: Teeth of the caudal margin long, thick, prominent and without any blunt process in the middle.
Fore-wing of the female adult	Fore-wing of the female adult
1. Size: 1·33 × ·61 m. m. Breadth proportionately greater than *D. kirkaldyi*.	*1. Size*: 1·08 × 0·42 m. m. Breadth proportionately less than *D. citri*.
2. Radial sector: Curvature sharp and greater than in *D. kirkaldyi*.	*2. Radial sector*: Curvature slight and gradual.

IX. Summary.

A full list of the plants so far recorded as the food plants of *D. citri* is given, but this list requires careful revision. From field observations, cross inoculation experiments and examination of the material available, it has been established that, in the Punjab, *D. citri* does not feed on any plant other than *Citrus* spp., and can not be bred under experimental conditions on *Jasminum sambac*, *Melia azedarach*, *Punica granatum*, *Psidium guava*, *Ficus religiosa*, *Pyrus communis*, *Aegle marmelos* and *Murraya exotica*. It has been established that the species attacking *Jasminum* and *Hiptage* is *D. kirkaldyi*, which cannot be bred on *Citrus*. The two species, although resembling in their general shape, are distinct in their food preference.

A comparative statement of the distinguishing characters of *D. citri* and *D. kirkaldyi* is given.

* Male pupae are smaller, average measurements being :—
D. citri —1·18×0·88 m. m. *D. Kirkaldyi*—0·93×0·71 m. m.

DIALEURODES CITRI, ASHM.

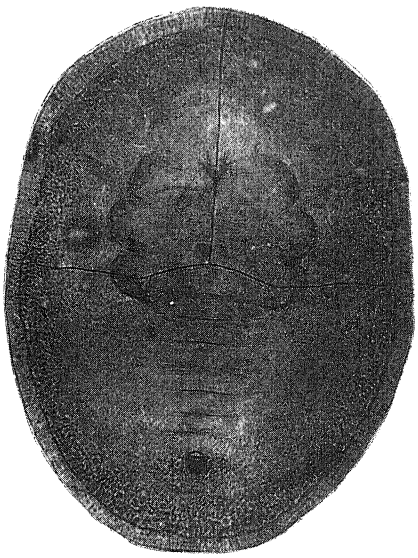


Fig. 1.—Pupa.

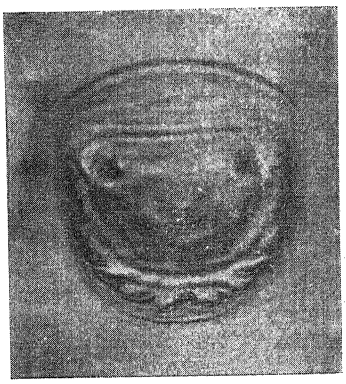


Fig. 2.—Vasiform orifice of the pupa.

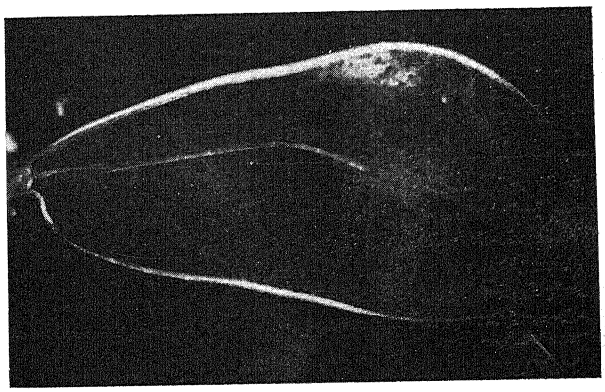


Fig. 3.—Fore-wing of the female.
(*dark ground illumination*)

DIALEURODES KIRKALDYI, KOT.

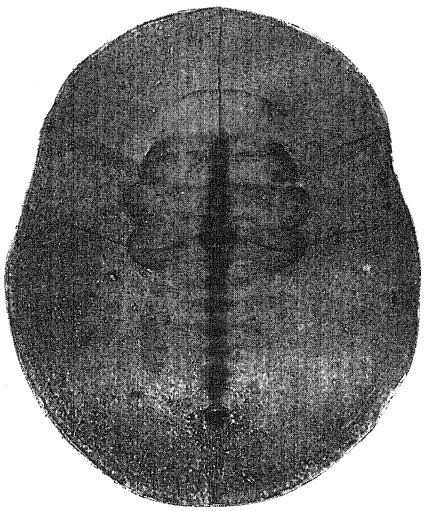


Fig. 4.—Pupa.

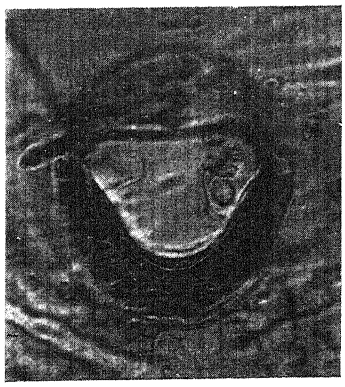


Fig. 5.—Vasiform orifice of the pupa.

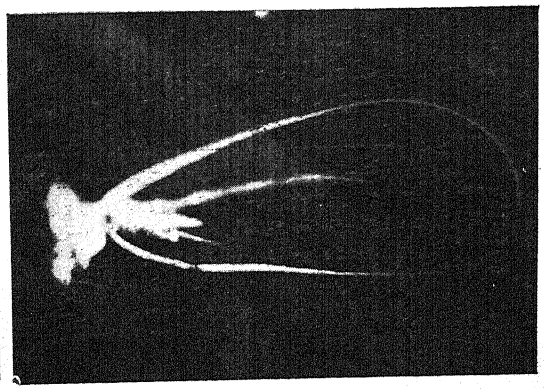


Fig. 6.—Fore-wing of the female.
(*dark ground illumination*)

REFERENCES.

1 Berger, E. W. (1908) . . . . White fly studies in 1908. Bull. No. 97. Fla. Agric. Exp. Sta.

2 ——————— (1910) . . . . White Fly control. Bull. No. 103. Fla. Agric. Exp. Sta.

3 Fletcher, T. Bainbrigge (1914) . . Some South Indian Insects.

4 ——————— (1920) . . . . . Rep. Pro. 3rd. Ent. Mtg., Pusa.

5 Gossard, H. A. (1903) . . . . White Flies (*A. citri*). Bull. No. 67. Fla. Agric. Exp. Sta.

6 Lamba, Karam Singh (1931) . . A contribution towards our knowledge of the Aleyrodidae (White Flies) of India. Mem. Dep. Agric. India. Vol. XII, No. 1, Pusa.

7 Misra, C. S. (1923) . . . . . "The Citrus White Fly, *Dialeurodes citri* in India and its parasite, together with the life history of *Aleyrodes citri*, n. sp. Rep. Proc. 5th. Ent. Mtg. Pusa.

8 Morrill, A. W. & Back, E. A. (1911) . White Flies injurious to Citrus in Florida. Bull No. 92, U. S. Dept. Agric. Washington.

9 Quaintance, A. L. (1900) . . . Contribution towards a monograph of the American Aleyrodidae. Tec. Sr. No. -8. U. S. Dept. Agric.

10 ——————— (1907) . . . . The more important Aleyrodidae infesting Economic plants. Tec. Sr. No. 12. Part 5th. U. S. Dept. Agric.

11 Quaintance, A. L. and Baker A. C. (1913) (1914). Agric. Classification of the Aleyrodidae. Parts I & II. Tec. Sr. No. 27. Parts, I & II. U. S. Dept.

12 ——————— (1917) . . . . . A contribution to our knowledge of the White Flies of the Sub-family Aleyrodinae (Aleyrodidae). No. 2156, Vol, 51, pp. 335, 445, Pl. 32, 77, Washington.

13 Woglum, R. S. (1913) . . . Report of a trip to India and the Orient in search of the Natural enemies of the Citrus White Fly. Bull. No. 120 U. S. Dept. Agric. Bu. Ent. Washington.

THE INHERITANCE OF CHARACTERS IN RAGI, *ELEUSINE CORACANA* (GAERTN.), PART VI. †

EARHEAD SHAPES.*

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(With Plates XVII—XIX.)

EARHEAD AND SPIKES.

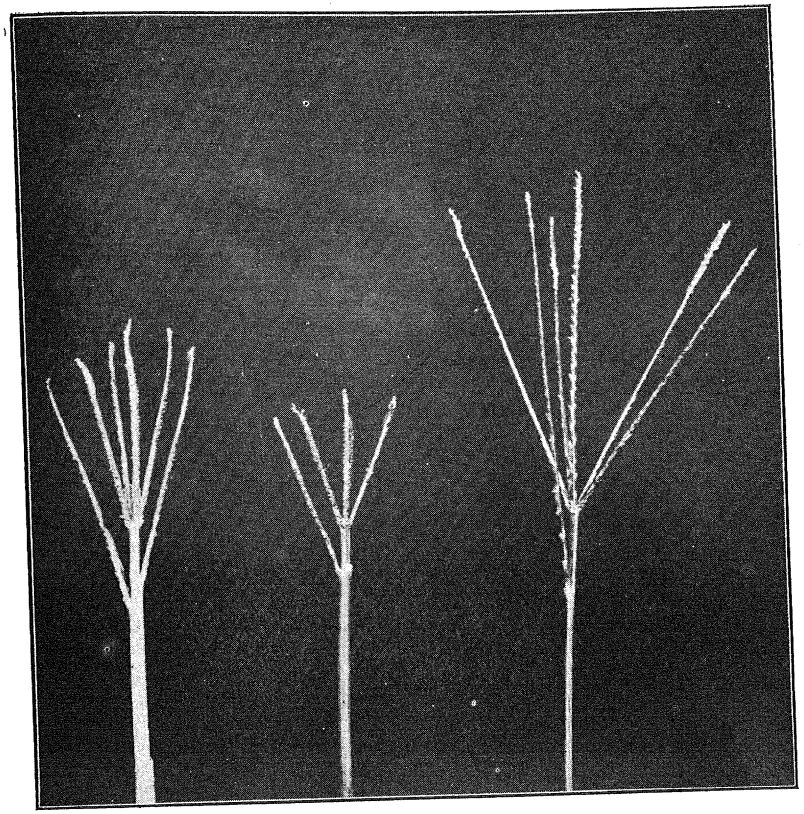
The earhead of *ragi* is a terminal whorl consisting of digitate spikes radiating from the apex of the culm with an almost constant single spike a little lower down, probably indicative of a second whorl. For the sake of convenience the spikes of the end whorl will hereafter be designated fingers and the odd lower one, the thumb.

SPIKELETS AND FLORETS.

Each of the spikes consists of two rows of sessile spikelets alternately attached to the underside of a flattened rachis. The florets are distichously arranged in the spikelets.

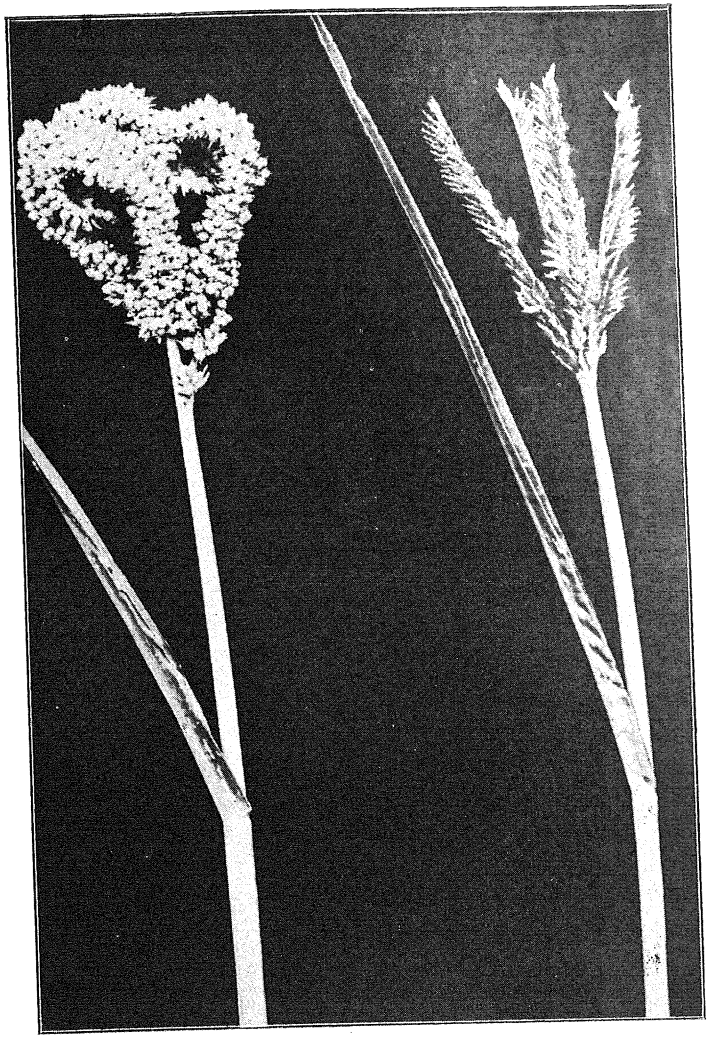
* Paper read at the 16th Session of the Indian Science Congress held at Madras, 1929 and supplemented with further data.

† Part I in August 1931 number and Parts II to V in the October 1931 number of the *Indian Journal of Agricultural Science.*



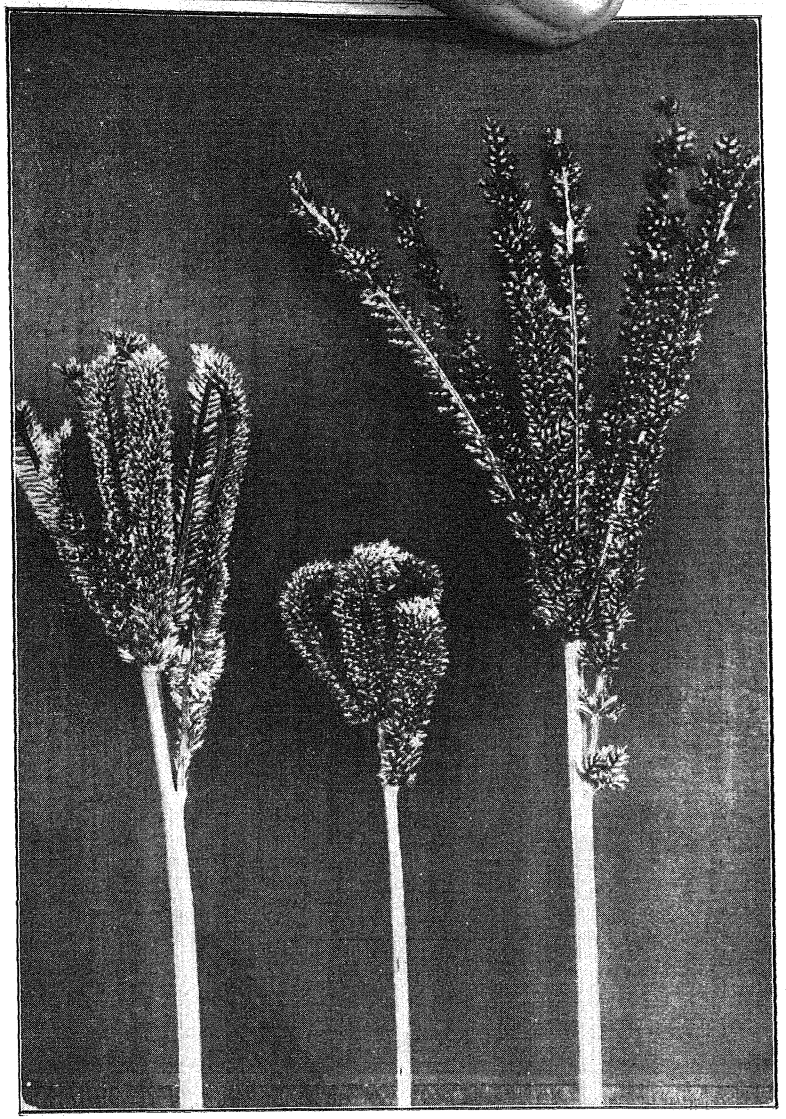
Top-curved. Incurved. Open.

Fig. 1. Panicle shape – spikelets removed.



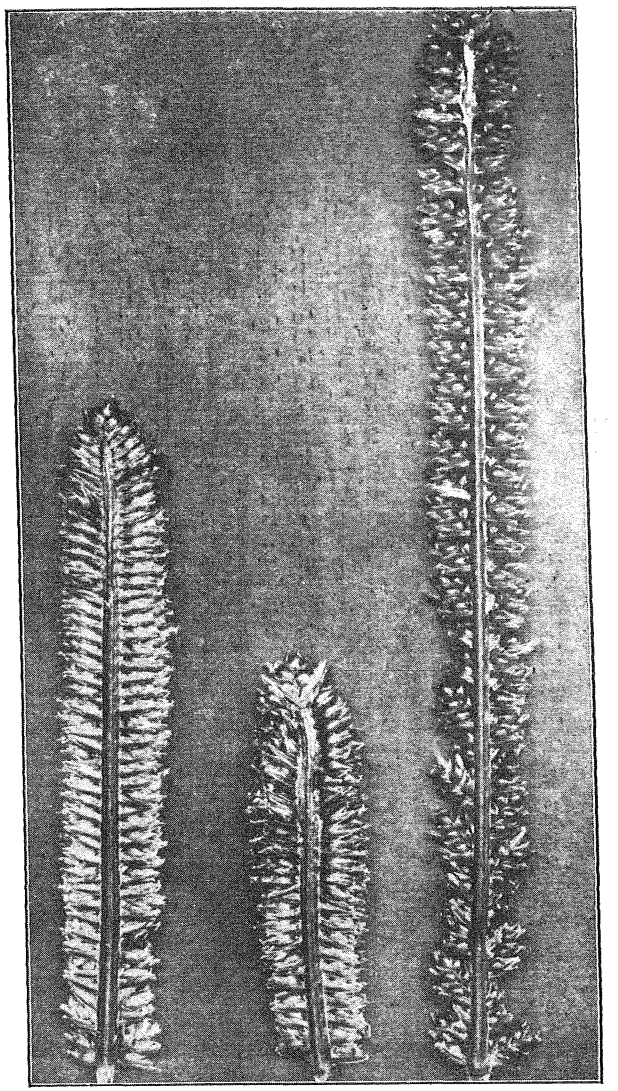
Fertile. Sterile.

Fig. 2. Panicle shape—fertile & sterile.



Top-curved. Incurved. Open.

Fig. 1. Panicle types in Ragi.



Top-curved. Incurved. Open.

Fig. 2. Panicle types—rear view of spikes.

THE EARHEAD TYPES.

There are three readily recognisable head shapes in *ragi*, namely, the Top-curved, the In-curved, and the Open (Plate XVII, fig. 1). The commonest of these in Madras is the In-curved. Next comes the Top-curved and next the Open.

In the In-curveds, the fingers are short and curve in and practically close up the central hollow giving the earhead an obovate shape.

In the Top-curveds, the curved fingers are longer with the result that they retain the central hollow.

In the Opens, the fingers are the longest and gape out and present a characteristic funnel shaped appearance. On drying they curve out slightly.

The earhead shapes show best in about the milky stage of the setting of the grains and should be read at that time on primary heads. The thumbed heads are the most reliable for these readings.

HEAD SHAPES AND FINGER LENGTHS.

Measurements on the pure lines at the Millet Breeding Station show out the most frequent finger lengths of the In-curved from 4 to 7 cm., of the Top-curved 5 to 10 cm., and of the Opens 8 to 15 cm.

NUMBER OF SPIKELETS IN THE FINGER.

The number of spikelets in a finger is slightly greater in the main season than in the summer season. The three types of earheads, their varying finger lengths notwithstanding, present no very marked differences in the number of spikelets on a finger in a season, though the Top-curveds give a slightly higher and the Opens a slightly lower number than the average. The average number of spikelets in a finger is 67 from over two hundred readings in 4 seasons, 2 main and 2 summer. They are distributed as follows :—

	Top-curveds	In-curveds	Opens
Main	73	72	67
Summer	66	63	59

ARRANGEMENT OF SPIKELETS ON THE FINGERS.

In the curved earheads, the heads lose their curving when the spikelets are removed. So also in the case of sterility (Plate XVIII). The spikelets are

attached to the underside of the rachis and to a rear view present in their close packing a series of edges characteristic of the curved heads. In the Opens the wider spacing of the spikelets presents a partial frontal view of these and gives the Open fingers their characteristic jagged outline in spikelet disposition (Plate XVII, fig. 2).

In all the three groups the spikelets increase in density towards the top. The average density of spikelets per centimeter length of the whole finger is as follows:—Top-curved 9·5, In-curved 11·8, and Open 6·7, the corresponding averages of the top-half only being 11·4, 12·9, and 7·9.

Head shape—Due to Mendelian factors.

A study of a number of pure lines at the Millet Breeding Station, Coimbatore, together with the pursuit of the history of a few natural crosses, makes it certain that the measureable length and visible curving of the earheads are due to definite Mendelian factors.

Two factors seem to be at work. The first, a factor for "density" (designated by the symbol Q) seems to be responsible for the densely disposed spikelets in the In-curveds and Top-curveds. The presence of this factor marks the Curved as broadly separated from the Open. In the Opens this factor is absent, resulting in a comparatively lax disposition of the spikelets and a lenghthening out of the fingers.

The second, a factor E for "elongation" seems to operate on both the Curveds and Opens. Its presence makes Top-curveds of the In-curveds and marks the Long Opens from the Short Opens.

The Top-curveds have thus both the factors in them and are separable from the In-curveds by a visible central hollow and a touching—not overlapping—of the fingers.

The In-curveds have the factor for Density alone. They lack the factor for Elongation. In them the denseness manifests in an incurving and an almost wiping out of the central hollow.

The Opens lack the factor for Density and as a result have spikelets laxly disposed. They are therefore long and hence open out. There seem to be two practically inseparable genetic groups in the Opens, those with and those without the factor for Elongation. Long Opens and Short Opens are difficult of separation in the absence of aids like central hollow and finger overlapping, which in the Curveds, help to mark out the Top-curveds from the In-curveds.

The nett result of this manifestation of characters in inheritance, is the resultant unmistakable 9 : 3 : 4 ratios of Top-curved, In-curved, and Open, that have been obtained in very many families—from both natural and artificial crosses, at

the Millet Breeding Station. The history of one of these big families, E. C. 500, typical of this experience, is presented below.

CLAN E. C.* 500.

Clan E. C. 500 has proved an important family in the history of *ragi* work at the Station. It was the second generation of an F_1 which had Top-curved fingers, spotted in the year 1925, out of an In-curved mother. In segregation, a 9 : 3 : 4 ratio of Top-curved, In-curved and Open was obtained (Table I). From this, 6 Top-curveds, 4 In-curveds and 4 Opens were carried forward and a third generation raised. In this generation the Opens came pure, one of the four In-curveds segregated, and 5 of the six Top-curveds also segregated (Table II). At this stage, the issues were narrowed down to a separation of the Opens, and with this end 6 Top-curveds and 2 Opens form E. C. 746 and 6 In-curveds and 2 Opens form E. C. 752, both from Table II and segregating for these pairs of characters alone, were carried forward to a fourth generation.

In the fourth generation, the Opens came pure as expected and the range of finger lengths between the two groups of Opens is very suggestive and helps to demonstrate the possibility of separating the Long Opens from the Short Opens (difficult of separation as the one runs into the other) by mating them with the easily separable Top-curveds and In-curveds and extracting the allelomorphic recessive Opens (Table III). The second point of interest in this fourth generation is a magnified repetition of the preponderence of segregating Top-curveds and pure In-curveds, noticed in the third generation itself. A detailed pursuit of this preponderence was made and a separation attempted among the Top-curveds and the In-curveds to find out if there had been any unconscious selective picking of seed material from the segregates. A closer examination of the Top-curveds and the In-curveds in segregating families indicated a separability of these into typical Top-curveds and In-curveds and their two heterozygous forms, in which the fingers showed a tendency to loosen out, showing in the case of Top-curveds a crater to the central hollow and in the case of the In-curveds an approach to the Top-curveds, in the disposition of the fingers. It is this approach to the Top-curveds that resulted in the unconscious selection of the obvious In-curveds to be sure that the heterozygous In-curveds with a little crater are not selected with the risk of thus selecting Top-curveds instead of In-curveds. This accounts for the paucity of heterozygous In-curveds among the In-curved selections. In the case of the Top-curveds there are certain lengths and phases of the manifestation of their character which approach the In-curveds in shape, and in choosing the markedly visible Top-curveds, there seems to have been an equally unconscious selection of heterozygous

* E. C. is an abbreviation for *Eleusine coracana*.

individuals. A separation of the homozygous from the heterozygous cannot with ease and certainty, be made in mere counts. Nevertheless, to elucidate the above points 24 selections—12 Top-curved and 12 separated as Top-curved' (on the basis of this central loosening—Plate XIX) were carefully chosen and bred, and from the In-curveds a similar 10 and 10. Their behaviour in the fifth generation (Tables IV and V) confirms this unconscious selection manifested in the previous tables.

TABLE I.

Clan E. C. 500 —F_1 and F_2 generations.

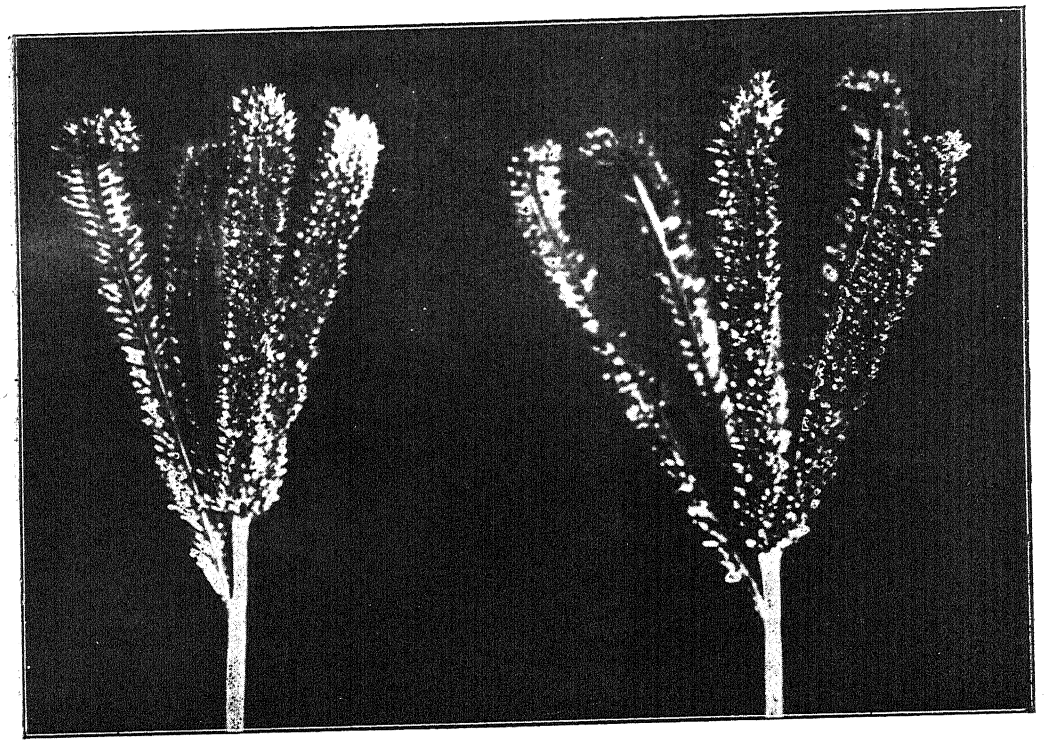
E. C. No.	Character	Finger length in cm.										Genetic constitution
		4	5	6	7	8	9	10	11	12	13	
167	*Inc.	..	6	30	..	..	..	..	..	..	..	QQee
F_1 167/a	†T. C.	..	..	..	..	..	..	F_1	..	..	..	QqEe
(Natural cross) 1925.												
F_2 500	T. C. 141	8	23	41	32	27	8	2	..	..	..	
	Inc. 40	2	23	11	4	..	..	..	..	..	..	
	Open 64	1	1	4	7	14	18	9	5	3	2	

* Inc.=In-curved.
† T. C.=Top-curved.

TABLE II.

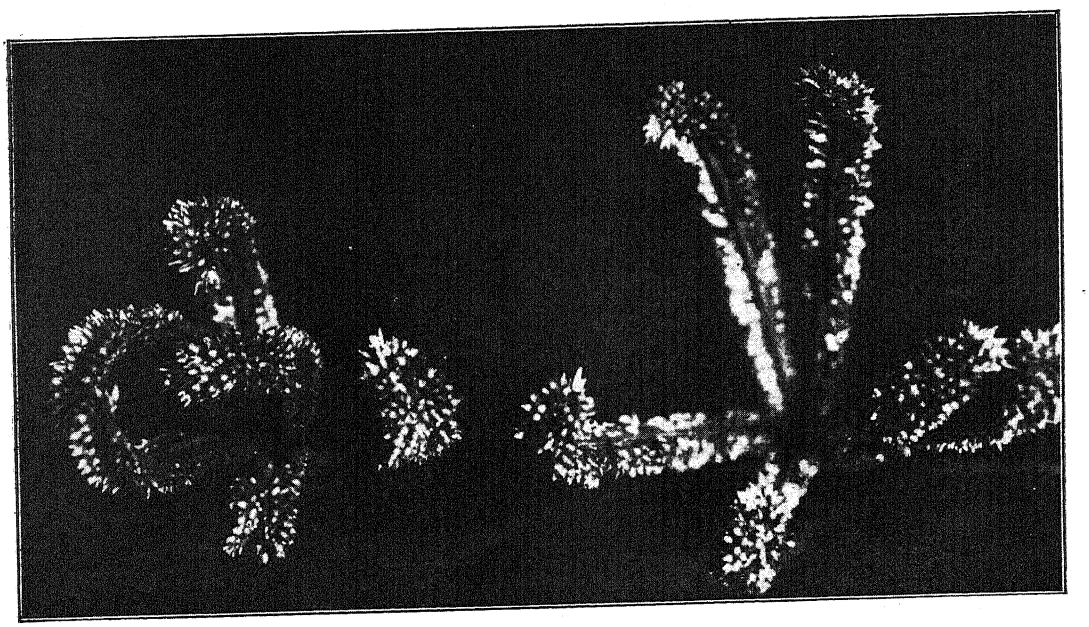
Clan E. C. 500—F_3 generation.

Family number E. C.	Character of selection	Panicle shape and numbers	Behaviour in F_3 Finger length in cm.													Genetic constitution
			4	5	6	7	8	9	10	11	12	13	14	15	16	
749	T. C. 7 cm.	T. C. 262	6	50	68	126	12	..	..	..	..	..	..	..	..	QQEE
746	T. C. 7 cm.	T. C 194	..	..	22	82	64	26	..	..	..	..	..	..	..	QqEE
		Open 62	..	..	..	..	..	3	15	23	15	5	1	..	..	
747	T. C. 7 cm.	T. C. 191	7	25	62	47	34	16	..	..	..	..	..	..	..	
		Open 49	..	..	..	1	5	8	18	10	5	2	..	..	..	



T. C. T. C.′

Fig. 1. Side view of panicle.



T. C. T. C.′

Fig. 2. Crater view of panicle.

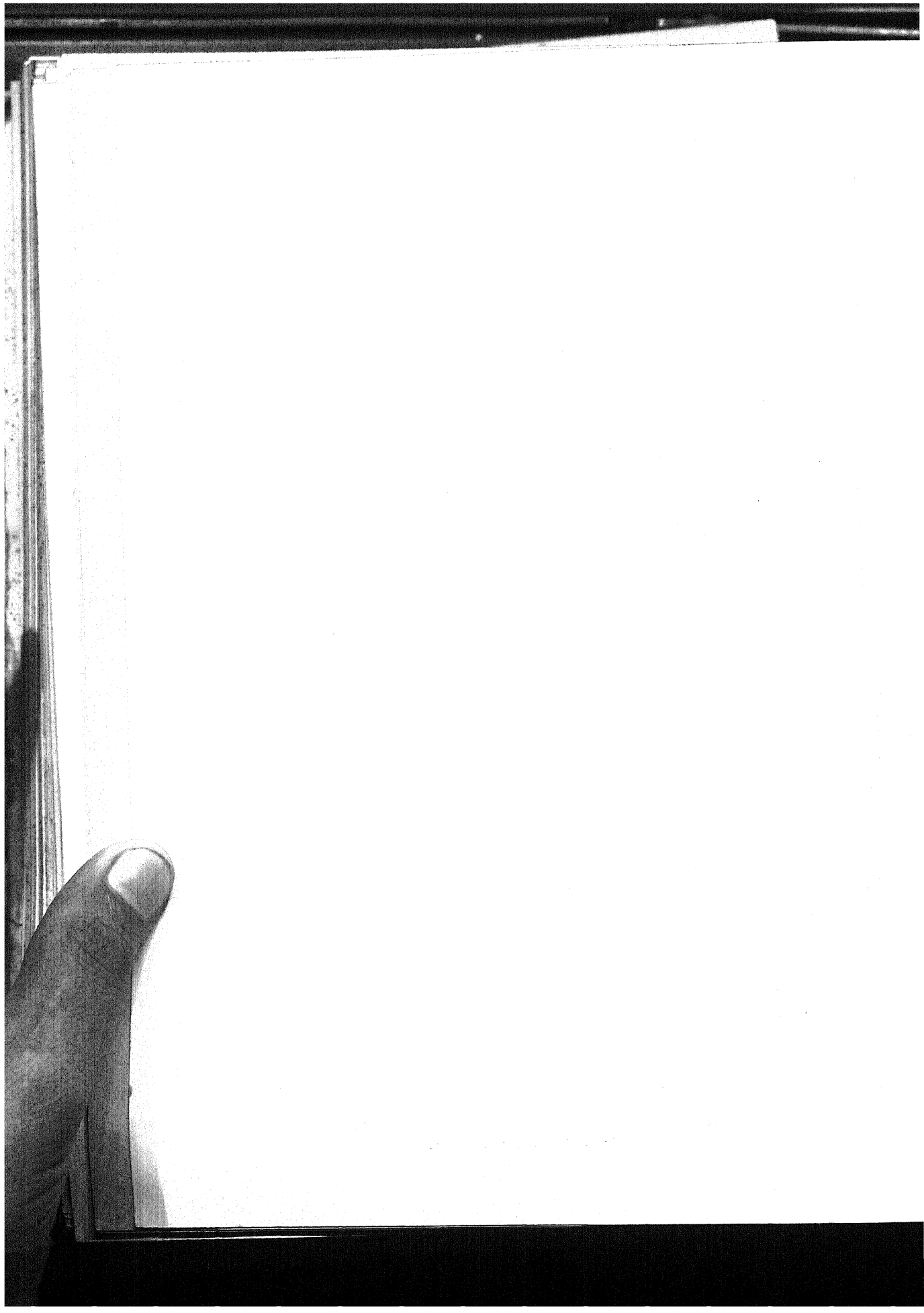


TABLE II—*contd.*

Clan E. C. 500—F_3 generation—contd.

Family number E. C.	Character or selection.	Panicle shape and numbers.	Behaviour in F^1 — Finger length in cm. 4	5	6	7	8	9	10	11	12	13	14	15	16	Genetic constitution.
744	T. C. 7 cm.	T. C. 137	..	5	72	43	11	6	..	..	..	..	..	..	..	QqEe
		Inc. 47	11	33	3	..	..	..	..	..	..	..	..	..	..	
		Open 59	..	..	..	5	9	9	16	9	10	1	..	..	..	
748	T. C. 7 cm.	T. C. 137	..	..	7	41	61	28	..	..	..	..	..	..	..	
		Inc. 58	..	19	38	1	..	..	..	..	..	..	..	..	..	
		Open 54	..	..	..	..	..	..	11	32	5	5	1	..	..	
745	T. C. 8 cm.	T. C. 162	..	..	11	52	73	25	1	..	..	..	..	..	..	
		Inc. 37	1	5	20	2	..	..	..	..	..	..	..	..	..	
		Open 68	..	..	..	..	2	6	12	20	16	3	..	..	..	
750	Inc. 5 cm.	Inc. 241	15	75	126	23	2	..	..	..	..	..	..	..	..	QQee
751	Inc. 5 cm.	Inc. 240	7	57	137	38	1	..	..	..	..	..	..	..	..	
753	Inc. 6 cm.	Inc. 246	1	69	123	42	11	..	..	..	..	..	..	..	..	
752	Inc. 6 cm.	Inc. 174	11	70	73	20	..	..	..	..	..	..	..	..	..	Qqee
		Open 67	..	..	..	1	6	10	19	23	8	..	..	..	..	
757	Open 9 cm.	Open 172	..	3	7	..	49	37	48	17	10	1	..	..	..	qqEE or qqee
755	Open 11 cm.	Open 262	..	..	..	..	2	7	34	86	78	40	10	5	..	
756	Open 11 cm.	Open 236	..	..	1	2	6	36	54	70	45	12	8	1	1	
754	Open 13 cm.	Open 270	..	..	..	2	4	16	38	63	77	49	18	3	..	

TABLE III.

Clan E. C. 500—F_4 generation.

Family number E. C.	Character of selection	Panicle shape and numbers	Behaviour in F_4 — Finger length in cm. 3	4	5	6	7	8	9	10	11	12	13	14	15	16	Genetic constitution.
	E. C. 746 Family																
958	T. C. 7 cm.	T. C. 100	..	..	..	15	75	10	..	..	..	..	..	..	..	..	QQEE
959	T. C. 7 cm.	T. C. 164	..	..	1	33	69	56	5	..	..	..	..	..	..	..	
		Open 54	..	..	..	..	..	3	11	22	10	4	2	1	1	..	
960	T. C. 7 cm.	T. C. 363	..	..	3	10	142	116	30	2	..	..	..	..	..	..	
		Open 115	..	..	..	..	..	3	19	39	22	26	4	2	..	..	
962	T. C. 7 cm.	T. C. 183	..	..	..	6	35	89	48	5	..	..	..	..	..	..	QqEE
		Open 61	..	..	..	..	..	..	..	4	20	20	11	3	3	..	
961	T. C. 9 cm.	T. C. 174	..	..	..	16	34	82	33	9	..	..	..	..	..	..	
		Open 62	..	..	..	..	..	..	1	6	22	25	7	1	..	..	
963	T. C. 9 cm.	T. C. 192	..	..	..	10	37	77	48	20	..	..	..	..	..	..	
		Open 56	..	..	..	..	..	..	2	5	16	16	9	5	1	2	
965	Open 9 cm.	Open 66	..	..	..	..	..	5	19	29	7	5	1	..	..	..	qqEE
964	Open 11 cm.	Open 90	..	..	..	..	1	8	33	33	10	5	..	..	..	..	
	E. C. 752 Family																
980	Inc. 5 cm.	Inc. 100	..	3	79	15	3	..	..	..	..	..	..	..	..	..	QQee
981	Inc. 5 cm.	Inc. 100	..	6	87	7	..	..	..	..	..	..	..	..	..	..	
982	Inc. 5 cm.	Inc. 100	..	4	92	4	..	..	..	..	..	..	..	..	..	..	
984	Inc. 7 cm.	Inc. 100	2	9	85	4	..	..	..	..	..	..	..	..	..	..	
985	Inc. 7 cm.	Inc. 100	3	21	76	..	..	..	..	..	..	..	..	..	..	..	
983	Inc. 7 cm.	Inc. 146	..	10	68	64	4	..	..	..	..	..	..	..	..	..	Qqee
		Open 60	..	..	..	..	4	16	16	13	10	..	..	1	..	..	
987	Open 7 cm.	Open 80	..	..	..	1	16	38	21	4	..	..	..	..	..	..	qqee
986	Open 11 cm.	Open 80	..	..	..	..	17	39	20	4	..	..	..	..	..	..	

TABLE IV.

Clan E. C. 500—F_5 generation. (E. C. 962 Family).

Family number E.C.	Character of selection		Behaviour in F_5											
			Panicle shape and numbers	Finger length in cm.										
				6	7	8	9	10	11	12	13	14	15	16
1223	T. C.	6cm.	T. C. 100	...	7	67	26	...	...	...	...	...	...	...
1224	T. C.	6cm.	T. C. 100	1	17	77	5	...	...	...	...	...	...	...
1225	T. C.	7cm.	T. C. 100	...	7	80	13	...	...	...	...	...	...	...
1226	T. C.	7cm.	T. C. 100	...	6	73	20	1	...	...	...	...	...	...
1227	T. C.	7cm.	T. C. 100	...	8	75	16	1	...	...	...	...	...	...
1228	T. C.	7cm.	T. C. 100	...	3	69	28	...	...	...	...	...	...	...
1229	T. C.	8cm.	T. C. 100	...	12	75	13	...	...	...	...	...	...	...
1230	T. C.	8cm.	T. C. 100	2	13	62	22	1	...	...	...	...	...	...
1231	T. C.	8cm.	T. C. 100	1	28	70	1	...	...	...	...	...	...	...
1232	T. C.	8cm.	T. C. 100	1	18	64	14	3	...	...	...	...	...	...
1233	T. C.	9cm.	T. C. 100	2	22	72	4	...	...	...	...	...	...	...
1234	T. C.	9cm.	T. C. 100	9	28	36	26	1	...	...	...	...	...	...
1236	T. C.	7cm.	T. C. 100	1	9	40	48	2	...	...	...	.	...	...
1235	T. C.	7cm.	T. C. 174	2	18	58	72	24	..	...	...	...	...	...
			Open 62	...	...	...	...	18	11	14	13	6	...	...
1237	T. C.	8cm.	T. C. 167	1	10	53	54	49	...	...	...	...	...	...
			Open 67	...	...	...	...	2	5	23	25	12	...	...
1238	T. C.	8cm.	T. C. 174	4	27	54	56	32	1	...	...	...	...	...
			Open 62	...	...	...	1	12	13	20	11	4	1	...
1239	T. C.	8cm.	T. C. 167	1	9	45	64	48	...	...	...	...	...	...
			Open 62	...	...	...	...	7	11	19	12	12	1	...
1240	T. C.	8cm.	T. C. 176	...	8	65	67	36	...	...	...	...	...	...
			Open 61	...	...	...	...	3	7	30	17	4	...	...

TABLE IV.—*contd.*

Clan E. C. 500—F_5 generation.—E. C. 932 Family.—contd.

Family number E. C.	Contractor of selection		Behaviour in F.											
			Panicle shape and numbers	Finger length in cm.										
				6	7	8	9	10	11	12	13	14	15	16
1241	T. C.	9cm.	T. C. 188	...	7	67	75	39	...	...	...	...	...	...
			Open 53	...	...	...	...	1	8	30	11	2	...	1
1242	T. C.	9cm.	T. C. 189	...	6	74	73	35	1	...	...	...	...	...
			Open 60	...	...	...	...	1	4	34	15	6	...	...
1243	T. C.	9cm.	T. C. 179	8	32	61	49	29	...	...	...	...	...	...
			Open 59	...	...	...	...	...	9	25	23	2	...	...
1244	T. C.	9cm.	T. C. 180	...	19	39	57	62	3	...	...	...	...	...
			Open 54	...	...	...	...	...	2	11	22	13	5	1
1245	T. C.	10cm.	T. C. 183	...	6	37	65	68	7	...	...	...	...	...
			Open 62	...	...	...	...	...	3	12	28	13	6	...
1246	T. C.	10cm.	T. C. 176	...	2	54	56	54	10	...	...	...	...	...
			Open 50	...	...	...	...	...	2	17	24	6	1	...
1247	Open	10cm.	Open 57	...	...	...	...	2	5	15	18	12	5	...
1248	Open	10cm.	Open 50	...	...	...	...	1	2	15	14	14	4	..
1249	Open	15cm.	Open 57	...	...	...	...	1	...	4	6	25	19	2
1250	Open	15cm.	Open 59	...	...	...	...	2	5	7	17	11	17	...

TABLE V.

Clan E. C. 500—F_5 generation—contd.—(*E. C. 983 Family*).

Family number E.C.	Character of selection	Behaviour in F_5											
		Panicle shape and numbers		Finger length in cm.									
				4	5	6	7	8	9	10	11	12	13	14
1251	Inc. 4cm.	Inc.	100	5	79	16	...	...	...	...	...	...	...	...
1252	Inc. 4cm.	Inc.	100	6	78	16	...	...	...	...	...	..	...	...
1253	Inc. 5cm.	Inc.	100	4	71	25	...	...	...	...	...	...	..	...
1254	Inc. 5cm.	Inc.	100	5	36	59	...	...	...	...	...	...	...	...
1255	Inc. 5cm.	Inc.	100	2	18	80	...	...	...	...	...		...	...
1256	Inc. 5cm.	Inc.	100	2	26	72	...	...	...	...	..	...	..	...
1257	Inc. 6cm.	Inc.	100	4	21	75	...	...	...	...	..	...	...	...
1258	Inc. 6cm.	Inc.	100	4	22	74	...	...	...	...	...	...	...	...
1259	Inc. 6cm.	Inc.	100	2	16	78	4	...	...	...	...	...	...	...
1260	Inc. 6cm.	Inc.	100	3	31	66	...	...	...	...	...	...	...	...
1262	Inc. 5cm.	Inc.	100	2	20	74	4	...	...	...	...	...	...	...
1261	Inc. 5cm.	Inc.	184	...	32	54	64	34	...	...	...	...	...	...
		Open	61	...	...	...	...	2	11	36	10	2	...	...
1263	Inc. 5cm.	Inc.	196	1	9	44	46	96	...	...	...	...	...	...
		Open	63	...	...	...	...	...	...	6	20	34	3	...
1264	Inc. 5cm.	Inc.	166	...	11	34	42	79	...	...	...	...	...	...
		Open	76	...	...	...	...	...	...	15	21	31	7	2
1265	Inc. 6cm.	Inc.	195	3	23	56	99	14	...	...	...	...	...	...
		Open	58	...	...	...	...	...	...	16	28	13	1	...
1266	Inc. 6cm.	Inc.	194	2	32	76	80	4	...	...	...	...	...	...
		Open	55	...	...	...	...	...	...	18	30	7	...	...
1267	Inc. 6cm.	Inc.	202	1	22	86	68	25	...	...	...	...	...	...
		Open	62	...	...	...	...	...	8	28	20	6	...	...
1268	Inc. 6cm.	Inc.	172	3	14	54	74	27	...	...	...	...	...	...
		Open	65	...	...	...	...	...	4	31	24	6	...	...
1269	Inc. 7cm.	Inc.	160	3	27	53	59	18	...	...	...	...	...	...
		Open	53	...	...	...	...	...	2	15	19	16	1	...
1270	Inc. 7cm.	Inc.	175	...	13	49	76	37	...	...	...	...	...	...
		Open	56	...	...	...	...	9	16	26	4	1	...	...
1271	Open 8cm.	Open	36	...	...	...	...	...	2	6	20	8	...	...
1272	Open 8cm.	Open	25	...	...	...	...	...	...	8	13	4	...	...
1273	Open 8cm.	Open	35	...	...	...	...	...	...	9	19	7	...	...
1274	Open 8cm.	Open	29	...	...	...	...	...	...	2	18	9	...	...

OPENS—LONG AND SHORT.

The individuality and constancy of the two groups of Opens—*viz.*, Long-opens and Short-opens—was sought to be further demonstrated as follows :—

E. C. 1273, a Short-open extracted from E. C. 983 of Table III and E. C. 1250, a Long-open extracted from E. C. 962 of Table III, were each grown side by side in 1929 summer season and duplicated. Each of the four strips had a population of 150. The range of the finger lengths of the four populations is tabulated below—

1929 Summer season.

Selection No. E.C.	Parental finger length in cm.	Finger lengths in cm.												
		7	8	9	10	11	12	13	14	15	16	17	18	19
1273 Short-open eeqq .	11	...	15	61	57	17	...	...	...	...	...	...	...	...
1250 Long-open EEqq	13	...	...	...	1	3	3	28	30	52	19	11	2	1
1273	...	2	23	50	63	11	1	...	...	...	...	...	...	...
1250	..	...	...	...	...	1	...	8	26	60	28	17	8	2

The range of the two opens is markedly different. The mean length of the 300 Short-opens is $9{\cdot}457 \pm {\cdot}033$ and of the 300 Long-opens $14{\cdot}973 \pm {\cdot}056$, a significant difference.

In the next season (1929 main) 6 selections one in each of Long-opens and Short-opens in the three over-lapping lengths at 10, 11 and 12 cm. were bred and the range of their finger lengths is given below :—

1929 main season.

Selection No. E.C.	Parental finger length in cm.	Finger lengths in cm.										
		8	9	10	11	12	13	14	15	16	17	18
1273/a	10	...	10	69	64	7	...	...	...	...	...	...
1250/a	10	...	...	...	4	29	42	33	29	8	2	3
1273/b	11	2	27	61	46	13	1	...	...	...	...	...
1250/b	11	...	...	4	14	34	41	29	22	4	2	...
1273/c	12	...	15	60	60	11	4	...	...	...	...	...
1250/c	12	...	...	...	...	27	46	49	24	4	...	...

The sameness of the reading of the selection notwithstanding, the clear tendency of the individual to conform to the parental range is obvious.

Artificial crosses.—16 artificial crosses (Crosses E. C. IV to IX, XII to XIV, XL, XLI, LVII, CXII, CXIII, CXXVI, CXXXI) were made with the background of the knowledge supplied by Clan E. C. 500 and bred through third and fourth generations. All these families repeat and confirm the behaviour of Clan E. C. 500 already presented in detail.

Purple pigmentation and panicle shapes.—The factors determining panicle shape seem to be independent of those responsible for purple pigmentation, [Rangaswami Ayyangar and Krishna Rao, 1931] as the behaviour of the following families in segregation will show. (Table VI.)

TABLE VI.

Purple pigmentation and panicle shape.

Family number E.C.	Panicle groups		Distribution in pigmentation groups		
			Purple	Light purple	Green throughout
1184	Top-curved	197	157	...	40
	Open	65	49	...	16
			206	...	56
807	Top-curved	193	...	147	46
	In-curved	62	...	46	16
			...	193	62
1199	Top-curved	198	108	43	47
	In-curved	73	42	12	19
			150	55	66
829	Top-curved	151	112	...	39
	In-curved	42	32	...	10
	Open	71	65	...	16
			199	...	65
634	Top-curved	95	59	11	18
	In-curved	26	11	5	10
	Open	35	24	5	6
			94	28	34

It will be noticed that families E. C. 807 and 1199 segregate for the E factor only, being pure for Q.

SUMMARY.

There are two broad groups of panicle shape in *Ragi—Eleusine coracana* (Gaertn.),—those in which the digitate spikes of the inflorescence curve in and those in which they are open.

A factor for Density designated Q, responsible for a close packing of spikelets on the rachis, is present in the curveds and absent in the opens.

The curveds are separable into In-curveds and Top-curveds. In the In-curveds the spikes curve in. The Top-curveds are longer than the In-curveds and in them, only the tops of the spikes curve. A second factor E determines the elongation of the rachis and separates the Top-curveds from the In-curveds.

The E factor is present in the Opens also and its presence or absence gives rise to two groups, the Long-opens and Short-opens. Their separation is difficult but their existence and individuality could easily be demonstrated from segregates with Top-curveds and In-curveds respectively.

Factors Q and E are independent of the factors for plant purple pigmentation.

REFERENCES.

Rangaswami Ayyangar G. N. and Krishna Rao P. (1931). *Ind. J. Agric. Sci. I*, 434.

INHERITANCE OF CHARACTERS IN SORGHUM, I.

CHLOROPHYLL DEFICIENCIES.*

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(With Plates XX-XXI).

LETHAL PALE GREEN.

At the Millet Breeding Station, Coimbatore, in the course of breeding work on sorghums, a case of Chlorophyll deficiency was experienced in March 1927. In two selections it was noticed that the seedlings were segregating into normal green and pale green (Plate XX, fig. 2.). This was clearly noticeable the sixth day after sowing. On the eighth day the leaf tips of the pales started shrivelling up. By about the twelfth day after sowing all the pales were dead. Counts taken of the dead ones in the two families gave the following figures :—

Selection No.	Green seedlings	Pale seedlings
A. S. 1554	138	36
A. S. 1556	276	131

* A paper read at the Seventeenth Annual Meeting of the Indian Science Congress.

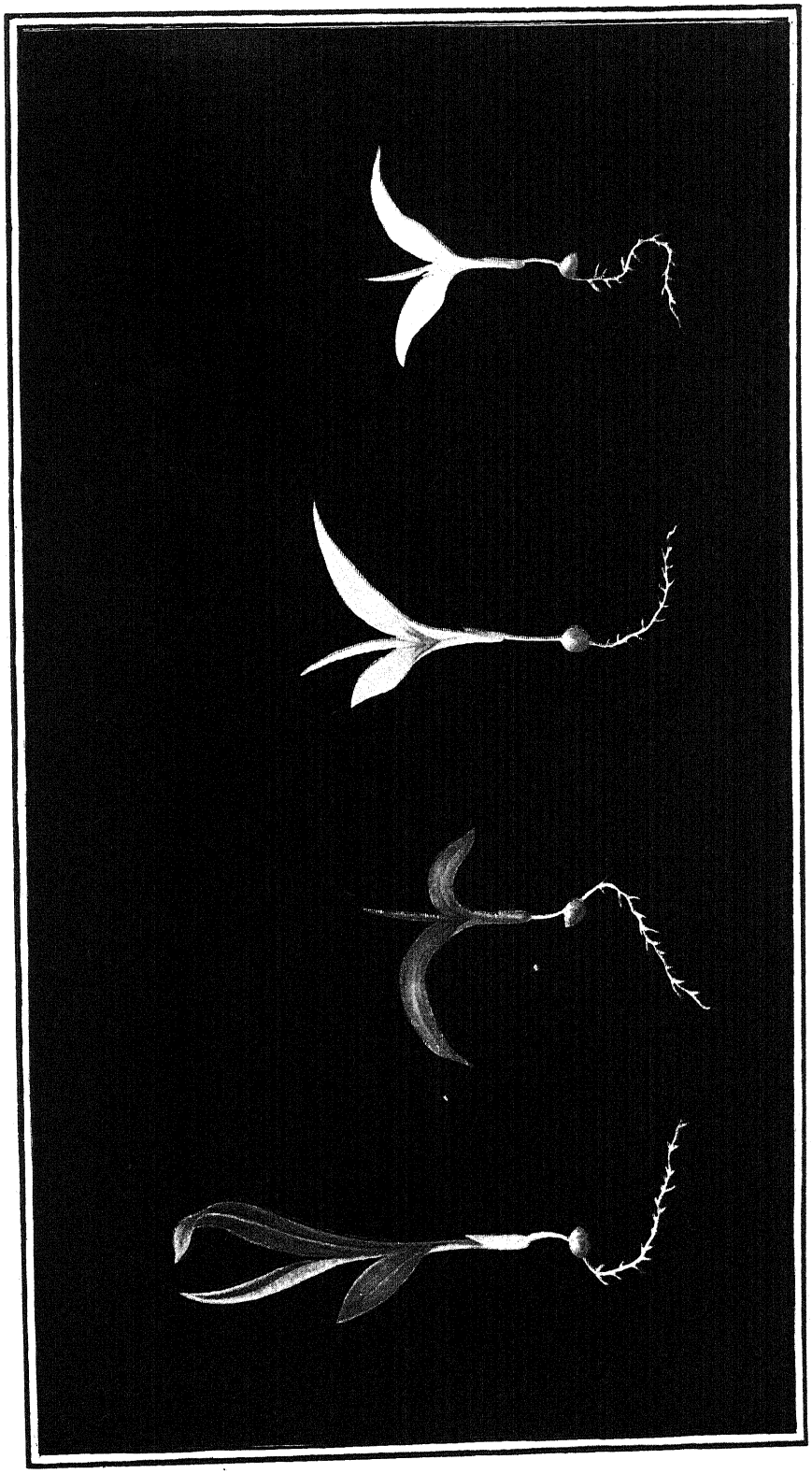
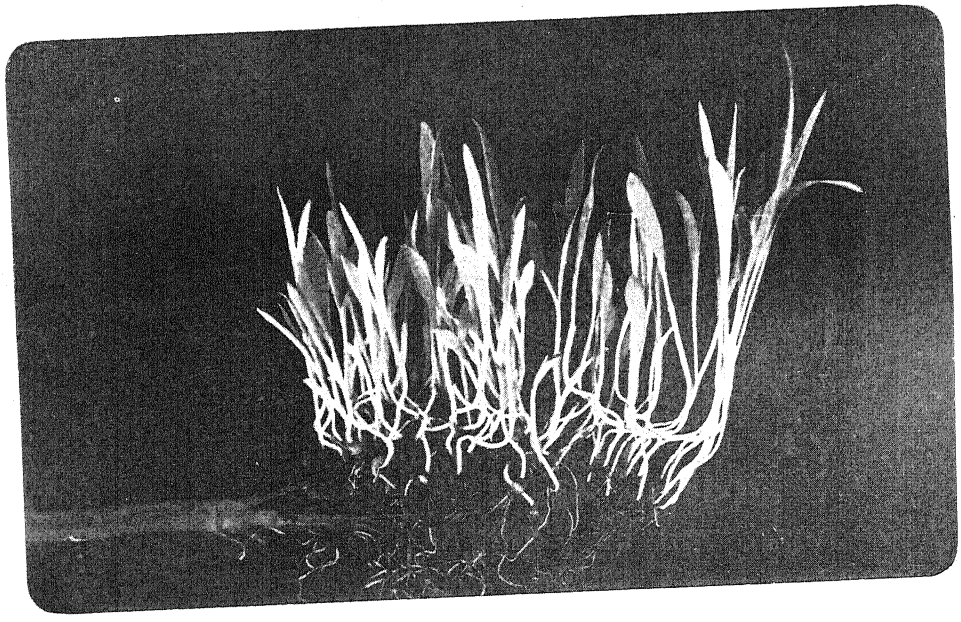

4
3
2
1

PLATE XXI.



Further selections were taken from these two families and the following table shows the seedling behaviour on germination :—

Family No.	No. of heads germinated	No. of heads showing segregation	No. of heads pure green
A. S. 1554	16	10	6
A. S. 1556	30	20	10

The thirty segregating earheads gave a total of 3813 green seedlings and 1048 dying pales.

Similar experiences have been met with in two other unrelated families. In A. S. 1022 segregating for the same character twenty earheads were taken and twelve of them segregated again leaving eight pure. In A. S. 1370 out of nineteen heads germinated fourteen segregated and five were pure.

A single factor difference seems to determine the lethal pale greens from the normal greens.

Virescent whites.

A second type of albinism, represented by virescent whites was met with, segregating with the normal green. These virescents take on a very light green tinge, giving the first leaves a yellowish look on the white background (Plate XX fiig. 3.). Their life-history is similar to the pales recorded above.

In A. S. 781 in which family these were first noticed in March 1928, thirteen segregating earheads gave 6975 greens and 2419 virescent whites, proving the existence of a single factor difference between healthy greens and virescent lethals.

Pure albions.

A third deficiency of a complete kind, resulting in seedlings quite white in colour, with a life-history similar to the two deficiencies noted above was experienced in August 1927 (Plate XX, fig. 4 and Plate XXI). In this instance, with coleoptiles usually purple, the first leaves take on a violet tinge, later to pale up, bleach and die out. Selections of albino leaves showed an absence of plastids. This kind of chlorophyll deficiency is experienced commonest and has been met with and worked out in a number of families. In A. S. 1043, typical of this experience, fifty-five earheads were sown and of these thirty-seven have been recorded as segregates.

Lingering lethal pales.

A fourth kind of deficiency, different from the previous three in having a deffered lethal condition was noticed in April 1928. In this instance, the

initial separation of the lethals is not easy till about the tenth day when paleness sets in and there is a pull down in growth. The pales manage to grow to a height of six inches and live for nearly one and a half months, after which they wither and die. Only three leaves are produced during this time, the top one measuring about ten cm. in length. Healthy contemporaries manage, during a similar period of existence, to give five leaves, the top one being as long as forty cm.

Family No. A. S. 2149 is typical of this experience, met with in six families. Fifty earheads from this family when germinated gave thirty-four segregating for pales and sixteen pure for greens. The ratios in these thirty-four families are given in Table I.

TABLE I.

Family No. A. S. 2149. (*34 segregating ear heads*).

Head No.	No. of seedlings			Ratio
	Green	Pale	Total	
A. S. 2149	604	131	735	3 : 0·65
A. S. 2149/39	566	138	704	3 : 0·73
A. S. 2149/50	539	123	662	3 : 0·68
A. S. 2149/7	582	127	709	3 : 0·65
A. S. 2149/19	591	128	719	3 : 0·65
A. S. 2149/24	630	135	765	3 : 0·64
A. S. 2149/27	583	121	704	3 : 0·62
A. S. 2149/28	591	119	710	3 : 0·60
A. S. 2149/30	544	108	652	3 : 0·60
A. S. 2149/21	551	109	660	3 : 0·59
A. S. 2149/20	485	92	577	3 : 0·57
A. S. 2149/10	547	101	648	3 : 0·55
A. S. 2149/40	561	99	660	3 : 0·53
A. S. 2149/6	526	92	618	3 : 0·52
A. S. 2149/15	638	111	749	3 : 0·52

TABLE I—*contd.*

Family No. A. S. 2149. (34 segregating ear heads)—contd.

Head No.	No. of seedlings			Ratio
	Green	Pale	Total	
A. S. 2149/35 . . .	536	93	629	3: 0·52
A. S. 2149/38 . . .	597	103	700	3: 0·52
A. S. 2149/17 . . .	480	79	559	3: 0·49
A. S. 2149/36 . . .	520	85	605	3: 0·49
A. S. 2149/45 . . .	480	77	557	3: 0·48
A. S. 2149/22 . . .	603	95	698	3: 0·47
A. S. 2149/9 . . .	591	91	682	3 : 0·46
A. S. 2149/5 . . .	593	90	683	3: 0·46
A. S. 2149/8 . . .	570	86	656	3: 0·45
A. S. 2149/26 . . .	633	94	727	3: 0·45
A. S. 2149/37 . . .	621	93	714	3: 0·45
A. S. 2149/46 . . .	680	102	782	3: 0·45
A. S. 2149/33 . . .	485	69	554	3: 0·43
A. S. 2149/32 . . .	524	73	597	3: 0·42
A. S. 2149/49 . . .	593	84	677	3: 0·42
A. S. 2149/42 . . .	485	61	546	3: 0·38
A. S. 2149/14 . . .	627	62	689	3: 0·30
A. S. 2149/31 . . .	544	54	598	3: 0·30
A. S. 2149/2 . . .	636	62	698	3: 0·29
A. S. 2149/3 . . .	490	44	534	3: 0·27
Total .	19,222	3,200	22,422	Average 3: 0·50

It will be noticed that the ratio of greens to pales is 3 : 0·50 or 14·3 per cent. recessive. This ratio seems to be explicable along lines confirmatory of the surmise of Coulter [1925] in letting in a probable zygotic lethal in explaining disturbed 3 : 1

ratios in grain colours in maize. On this basis a 14·3 per cent. gives a cross-over value of 24·4 per cent., using the following formula of Coulter :—

$$\text{Percentage of cross-overs} = 100 \frac{\sqrt{\frac{4n}{3}} - 2\sqrt{\frac{n}{3} - w}}{\sqrt{\frac{4n}{3}}}$$

where n is the total population and w is the lingering pales.

In a sister family—A. S. 1660—twenty-eight heads germinated gave a total of 15,589 greens and 2,504 pales, in the ratio of 3 : 0·48, with a confirmatory cross-over percentage of 23·5.

Surviving pales.

A fifth grade of deficiency the only one of the surviving kind—is also present. This was recognised in August 1927. In Family No. A. S. 149, segregating into green and pales in the ordinary manner, it has been possible to breed out and fix types of greens and pales—A. S. 210 and A. S. 211. The living pales are characterised by reduced growth and later flowering.

Chlorophyll estimations.

Chlorophyll estimations of the various types of deficiencies with their relative surviving normal greens reveal an absence of chlorophyll in pure albinos, 5 per cent. in virescent whites, 40 per cent. in lethal pale green and 51 per cent. in lingering lethal pales.

Stray albinos.

In a number of other families albinos and occasionally pales that could be readily spotted have been met with, as few as two or three in a population of more than 500. In one such family with stray albinos forty-five ear-heads germinated gave none but greens proving an absence of regular segregation and supporting the mutational origin to these chlorophyll deficiencies.

Summary.

Five types of chlorophyll deficiencies in sorghum are described and recorded as simple recessives to the normal healthy green. Four of these types prove lethal of varying degrees. The fifth survives. In one type of deficiency, distorted 3 : 1 ratios have been set to the presence of a zygotic lethal factor.

Reference.

Coulter, M. C. (1925). *The Bot. Gaz.* **79**, 28-44.

SOME OBSERVATIONS ON THE CHARACTERS OF WILD RICE HYBRIDS.

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(With Plate XXII.)

The wild rice (*O. sativa* var. *fatua*) is found generally in the *beels* (large natural depressions), ditches and other low-lying waste lands in Assam. It is always self-sown and is the mainstay of the cattle of Surma Valley, specially during the rains when other fodder grasses become scarce. It also grows in the ditches adjacent to the experimental plots of the Government Rice Farm, Karimganj, where *aus* (autumn rice) and *sail* (high land winter rice) are mostly grown. Although it is liable to be intercrossed with the *sail*, no evidence of natural crossing with wild rice has been obtained in Assam, but in 1923, to study the segregation of wild characters, a few flowers of the cultivated variety, Latisail (S. 22), were crossed with pollen from a wild plant, but owing to the shedding habit of the grains in the wild rice no reciprocal crosses were tried.

The principal characters of two parent plants may be stated as follows :—

(*a*) *Wild rice.*—The wild rice is a strong growing coloured plant with spreading and creeping habit. Like the deep water *aman* rice, it grows well in deep water where the other parental variety (Latisail) will not grow at all. The panicle is erect and loosely branched. The inner glume is green with black tinge ultimately turning black before maturity. The apiculus is pink with a deep purple stigma. Both the stigma and the anthers are larger in size than the cultivated variety. The awns are very long (about four inches), red, brittle and serrated the like of which is seldom met with in the cultivated varieties of *sail* and *aus* in Surma Valley. The kernel is slender and red in colour. The grains, when mature, shed very easily and are consequently very difficult to collect.

(*b*) *Latisail.*—The cultivated rice. Latisail, is a green plant with erect habit and close ear. It grows in places where water does not rise above two feet and has only a trace of awns in a few grains towards the apex of the ear. Unlike the wild rice its grains are not liable to shed and will separate only after a good threshing.

The characters of the two parent plants studied in this species cross, together with those of F_1 generation may be tabulated as follows :—

Characters	Wild rice	Latisail	F_1
Leaf-sheath	Purple	Geeen	Purple
Pulvinus	Light purple	White green	Light purple
Ligule	Ditto	Ditto	Ditto
Margin of leaf	Purple	Green	Purple
Auricle	White green	White green	White green
Node	Purple	Green	Purple
Internode	Do.	Yellow green	Do.
Outer glume	White green	White green	White green
Inner glume (floral)	Green with black tinge ultimately black.	Green	Green with black tinge ultimately black.
Apiculus	Light purple	White green	Light purple
Awn	Awned (long awns)	Trace and short	Awned (long awns)
Stigma	Deep purple	White	Deep purple
Straw	Weak (spreading)	Strong (erect)	Weak (spreading)
Panicle	Spreading	Close	Spreading
Inner glume (mature)	Black	Yellow	Black
Kernel (pericarp)	Red	White	Red

The above table shows that in the resulting hybrids the wild characters are dominant and the F_1 plants are like their wild parent. But the F_1 plants differed from the parent plants quite markedly both in vigour and size as shown in Plate XXII, fig. 1. Only one F_1 plant was selected for the F_2 and 234 plants were obtained. As there was a number of morphological and agronomical factors involved in this cross, it was not possible to predict at the outset the nature of interaction of factors under Mendelian doctrine. To what extent the characters of the wild parent are segregated and recombined is shown from results of F_2 and F_3 generations as discussed below.

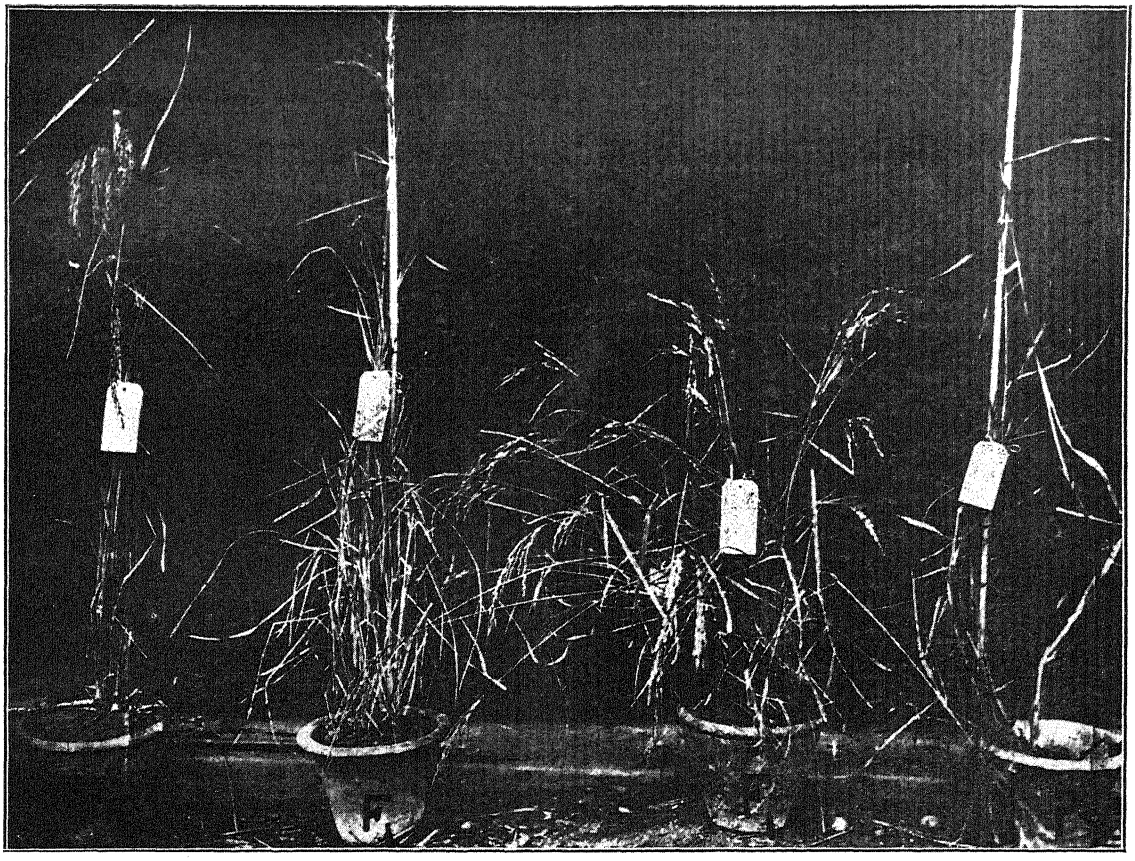


Fig. 1.—Parent plants, P $_1$(cultivated on the left and wild on the right) with two F_1 hybrids in the middle.



Fig. 2.—F_2 plants in the field. Most of the plants are spreading in their habit of growth except a few erect ones giving an approximate ratio of 3 : 1.

(1) *Leaf-sheath, pulvinus, ligule, margin of leaf and internode.*—The colour of the above characters segregated in F_2 in a simple Mendelian ratio of 3 : 1 as shown below.

	F_2 Generation		Total
	Dominant	Recessive	
Observed frequency	188	46	234
Expected „	175·5	58·5	234
Observed ratio	3·21 :	0·79	...
Expected „	3 :	1	...

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{12{\cdot}5}{4{\cdot}40} = 2{\cdot}8.$$

Although the fit is not quite good the F_3 results confirm the above ratio. In F_3 all the 46 green plants bred true in all cases and out of 188 purple plants, 49 bred true and the rest segregated as shown in the following table :—

F_2	F_3 segregations		
	Segregations	Observed	Expected
188 Purple	Pure	49	63
	Segregating	139	125
46 Green	Pure	46	46

(2) *Apiculus and stigma.*—The colour of both the characters behaved in a similar way and segregated in F_2 in a simple ratio of 3 : 1 as in the previous case.

	F_2 Generation		Total
	Dominant	Recessive	
Observed frequency	188	46	234
Expected „	175·5	58·5	234
Observed ratio	3·21 :	0·79	...
Expected „	3 :	1	...

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{12{\cdot}5}{4{\cdot}4} = 2{\cdot}8.$$

Although the fit is not quite good the F_3 results confirm the above ratio. In F_3 all the 46 green plants bred true in both the cases and out of 188 purple plants 54 bred true and the rest segregated as shown in the following table :—

F_2	F_3 segregations		
	Segregations	Observed	Expected
188 Purple	Pure	54	63
	Segregating	134	125
46 Green	Pure	46	46

(3) *Node.*—The colour of node segregated in the ratio of 3 : 1, the observed ratio being 2·96 : 1·04. This character was not noted in F_3. The observed and expected figures are noted in the table below :—

	F_2 Generation		Total
	Purple	Green	
Observed frequency	173	61	234
Expected „	175·5	58·5	234
Observed ratio	2·96 :	1·04	...
Expected „	3 :	1	...

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{2{\cdot}5}{4{\cdot}4} = {\cdot}57.$$ The fit is very good.

(4) *Auricle.*—The colour of the auricle is white green in both the parent plants as well as in F_1 generation, but in F_2 a new light purple colour appeared in 18 out of 234 plants giving an observed ratio of 14·77 white green : 1·23 light purple, thus approaching the theoretical ratio of 15 : 1. This indicates a case of double white green dominant and double light purple recessive factors [Mitra, Gupta and

Ganguli, 1928]. The colour of the auricle was not noted in F_3. The following table shows the segregation in F_2 generation :—

	F_2 Generation		Total
	White green	Light purple	
Observed frequency	216	18	234
Expected ,, ...	219·37	14·63	234
Observed ratio	14·77 :	1·23	...
Expected ,, ...	15 :	1	...

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{3{\cdot}37}{2{\cdot}5} = 1{\cdot}35.$$ The fit is good.

(5) *Outer glume and inner glume (florai).*—The colour of the outer glume and inner glume (floral) behaved like the auricle. But, as expected, the recessive colour did not breed true in F_3, which probably was due to some accidental natural crosses or the interaction of some other factors involved in the cross which could not be traced definitely.

	F_2 Generation		Total
	Green	Purple	
Observed frequency	208	26	234
Expected ,, ...	219·37	14·63	234
Observed ratio	14·22 :	1·78	...
Expected ,, ...	15 :	1	...

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{11{\cdot}37}{2{\cdot}5} = 4{\cdot}55.$$ The fit is not good.

(6) *Inner glume (mature).*—The colour of the inner glume (mature) of the wild rice is black and that of the cultivated type yellow. The black colour was

dominant in F_1 and it segregated in F_2 in the ratio of 9 black : 7 yellow, the observed ratio being 8·41 : 7·59 as shown in the table below :—

	F_2 Generation		Total
	Black	Yellow	
Observed frequency	123	111	234
Expected ,, ...	131·63	102·37	234
Observed ratio	8·41 :	7·59	...
Expected ,, ...	9 :	7	...

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{8·63}{5·12} = 1·68.$$ The fit is good.

The colour of the mature inner glume could not be noted in F_3.

(7) *Kernel (pericarp).*—The colour of the kernel segregates in a different way. The kernel of the wild plant is red and that of the cultivated white. The F_1 colour was red, but in F_2 another intermediate amber colour appeared in the ratio of 12 : 3 : 1 as shown in the table below :—

	F_2 Generation			Total
	Red	Amber	White	
Observed frequency . . .	174	43	17	234
Expected ,, . . .	175·5	43·87	14·63	234
Observed ratio	11·9	2·94 :	1·16	...
Expected ,, ...	12	3 :	1	...

$X^2 = ·407$. $P = ·82$. The fit is very good.

As the grains shed very easily with maturity the colour of the kernel could not be noted in F_3.

(8) *Straw.*—The most distinguishing character of the wild rice is the spreading habit of the straw which was dominant in F_1. In F_2 there were 184 spreading and 50 erect plants giving an approximate ratio of 3 : 1 (Plate XXII, fig. 2).

	F_2 Generation		Total
	Spreading	Erect	
Observed frequency	184	50	234
Expected ,,	175·5	58·5	234
Observed ratio	3·15 :	0·85	...
Expected ,,	3 :	1	...

$\frac{\text{Deviation}}{\text{Probable error}} = \frac{8·5}{4·4} = 1·93$. The fit is good.

In F_3 all erect plants bred true and out of 184 spreading plants 50 bred true and the rest segregated as shown in the following table :—

F_2	F_3 Segregation		
	Segregations	Observed	Expected
184 spreading	Pure	50	61
	Segregation	134	123
50 erect	Pure	50	50

(9) *Panicle.*—The panicle of the wild rice is distinctly spreading in habit while that of the cultivated is close. In the F_1 plant the wild character was dominant and in F_2 there were 148 plants with spreading and 86 with close ears giving an approximate ratio of 9 spreading : 7 close, the observed ratio being 10·12 : 5·88 as shown in the table below :—

	F_2 Generation		Total
	Spreading	Close	
Observed frequency	148	86	234
Expected ,,	131·63	102·37	234
Observed ratio	10·12 :	5·88	...
Expected ,,	9 :	7	...

$\frac{\text{Deviation}}{\text{Probable error}} = \frac{16·37}{5·12} = 3·2$. The fit is not good.

The F_3 characters could not be noted.

(10) *Awn.*—The awn character of the wild rice was dominant in F_1 and F_2. Apart from the awned and the intermediate (trace and short) characters another awnless character also came out in the ratio of 12 awned : 3 intermediate : 1 awnless, the observed ratio being 12·38 : 2·8 : ·82 as shown in the following table :—

	F_2 Generation			Total
	Awned	Intermediate	Awnless	
Observed frequency	181	41	12	234
Expected ,,	175·5	43·87	14·63	234
Observed ratio	12·38 :	2·8 :	·82	...
Expected ,,	12 :	3 :	1	...

X^2= ·832. P= ·66. The fit is very good.

In F_3 there were actually 112 awned, 68 awned and intermediate, 38 awned and awnless, 1 intermediate and awnless, 3 intermediate, and 12 awnless and consequently the deviation from the expected ratio is somewhat large.

It may also be mentioned here that the shedding habit of the grains which is always prominent in the wild rice was dominant in F_1 generation. In F_2 most of the plants were quite shedding in habit like the wild plant, while there were some with intermediate characters but none with the character of the cultivated parent plant. This was also the case in F_3.

The following will show the factor analysis of the plants the data of which have been presented before :—

Symbols	Characters for which factors are responsible	Ratios
C_c	Factor for colour of leaf-sheath, pulvinus, ligule, margin of leaf, internode, apiculus and stigma.	3 : 1
N_n	Factor for colour of node	3 : 1
A_1a_1	Complementary factors for colour of auricle	15 : 1
A_2a_2		
G_1g_1	Complementary factors for colour of outer glume and inner glume (floral).	15 : 1
G_2g_2		
H_1h_1	Complementary factors for colour of inner glume (mature)	9 : 7
H_2h_2		
P_1p_1	Complementary factors for colour of kernel (pericarp)	12 : 3 : 1
P_2p_2		
Ss	Factor for spreading habit of straw	3 : 1
E_1e_1	Complementary factors for spreading habit of panicle	9 : 7
E_2e_2		
W_1w_1	Complementary factors for length of awn	12 : 3 : 1
W_2w_2		

The genetic constitution of the two plants so far as the above characters are concerned may, therefore, be stated as follows :—

Wild plant—CCNNA$_1$A$_1$	a$_2$a$_2$	G$_1$G$_1$	g$_2$g$_2$	H$_1$H$_1$	H$_2$H$_2$
P$_1$P$_1$	P$_2$P$_2$	SS	E$_1$E$_1$	E$_2$E$_2$	W$_1$W$_1$	w$_2$w$_2$
Latisail (cultivated variety)— ccnn	a$_1$a$_1$	A$_2$A$_2$	g$_1$g$_1$	G$_2$G$_2$	h$_1$h$_1$	h$_2$h$_2$
p$_1$p$_1$	p$_2$p$_2$	ss	e$_1$e$_1$	e$_2$e$_2$	w$_1$w$_1$	W$_2$W$_2$

In fact, the F_2 plants showed a wide range of variation which was mainly due to the result of Mendelian segregation in recombination of characters of which there were many. The divergence in F_2 plants was so wide that it was rather difficult to trace the analysis of a factor or factors involved in this species cross. Moreover, the diffierent types in F_2 represented a continuous series of intermediates as a result of a cross between two species with characters lying in two extremes.

In F_3 generation there was again a split among the individuals and out of 234 plants none bred true in all the characters involved and they were mostly heterozygous, while a few bred true in regard to some particular characters, such as the colour of individual parts. The indication of a Mendelian segregation was evident and as a matter of fact the figures very nearly approached the theoretical ratios in several cases, but in a few others the range of segregation was, of course, very wide. It may also be pointed out here that even in F_3 out of 9,360 plants not a single plant appeared which could practically be indentified with the cultivated type, which was recessive in character.

Some of the selected plants were continued to get some pure strains but in all cases they split up to F_5. In F_6 five plants with itermediate characters were found to be pure.

Evidently, if the results of such a cross between two species are to be explained on the basis of Mendelian heredity it must be assumed that the two species possess relatively a large number of factor differences in relation to colour (individual parts), habit (straw and panicle), size of awn, and all the divergences that resulted in the cross are mainly due to peculiar factor interactions.

REFERENCE.

Mitra, S. K., Gupta, S. N. and Ganguli, P. M. (1928). *Mem. Dept. of Agric. Bot. Ser.* Vol. XV, No. 4, pp. 99--100.

STUDIES IN INDIAN BRASSICAE, I.

STERILITY AND SELECTIVE POLLEN TUBE GROWTH.

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(With Plate XXIII)

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I. Introduction.

A large number of samples of *Brassica* species commonly known as *sarson* (*Brassica campestris*, L. var. *sarson*, Prain), *toria* (*Brassica napus* L. var. *dichotoma*, Prain), *rai* (*Brassica juncea*, Hook.), and *taramira* (*Eruca sativa*, Lamk) were collected by the Botanical Section, Pusa, from all over India in 1929-30 with the object of isolation of types and other investigations. The information regarding the vernacular names supplied by the different localities is very confusing. The same variety has been received from different localities with different names. Moreover, in some cases different provinces give the same name to varieties which are quite distinct from one another. The information regarding the botanical names of these species in the Indian works of Botany is also very confusing. The best available record is that of Prain [1898]. Some varieties under investigation in the Botanical Section, Pusa, are not dealt with by Prain.

A classification of the species with detailed descriptions of the group will form a subject of future publication.

It was found that yellow *sarson* (*Brassica campestris*, L. var. *sarson*, Prain); *rai* ordinary (*Brassica juncea*, Coss.); and *rai* young habit cabbage like (*Brassica rugosa* var. *cuneifolia*, Prain*) form seeds under bag, while the other varieties namely black *sarson* late (*Brassica campestris*, L. var. *oleifera*, Prain); *toria*† (*Brassica napus*, L. var. *dichotoma*, Prain); black *sarson* early‡ (*Brassica campestris*, sub. sp. *napus* var. *dichotoma*, Duthie and Fuller); *Burma rai* (*Brassica rugosa*, Prain); white *rai* (*Brassica alba*., Boiss); *asl rai* (*Brassica nigra*, Koch); and *taramira* (*Eruca sativa*, Mill.) do not form seeds under bag.

The present studies were undertaken with the object in view of gathering some evidence for pollen tube development in compatible and incompatible matings, and to find out the exact nature of sterility.

II. Résumé of previous studies.

Many species of hermaphrodite animals and plants exist in which a union between the male and the female gametes produced by the same individual is either difficult or impossible. Both the male and the female gametes are morphologically perfect in most of the cases, and are functional with the gametes of other individuals. This phenomenon is generally known as self-sterility. Other terms have also been proposed. Darwin [1876] gave the name "self-impotence", Loew [1895] called it "adynamandry", and Stout [1917] proposed the term "self-incompatibility".

Self-sterility is a widespread phenomenon, though its occurrence has been proved experimentally in a few cases only. Knuth [1906] has mentioned a list of cases, the majority of them well founded, indicating that among the angiosperms the condition is rather widely distributed. He gives a list of 134 self-sterile species representing forty-six families and including both monocotyledons and dicotyledons.

The investigations of different observers with regard to self-sterility and self-fertility of many plants have not infrequently given contradictory results. Knuth [1906] quotes, "that according to some authorities rape (*Brassica rapa*) is self-fertile, while according to others it is self-sterile; so that we must assume that self-sterility is a character that is not constant for all individuals of the same species, but varies with the locality and the individuals." Stout [1922] working on *Brassica pekinensis* Skeels finds marked range in seed production depending upon season.

It was early shown by Scott [1865], that pollen tubes are produced freely in the style of self-sterile plants after selfing. The only data on pollen tube growth

* Not given in the Kew Index. Detailed description given in Agric. Ledger, 51-78.

† and ‡ *Toria* and black *sarson* early differ in size and maturity. *Toria* variety is early dwarf while black *sarson* early variety is late tall as compared to *toria*.

bearing directly on the problems of self-sterility are those of Jost [1907] and of Correns [1912]. They found that when a self-sterile plant is pollinated with its own pollen, the tubes are emitted freely, but grow extremely slowly. They also showed that pollen tubes grow rapidly when cross-pollination is made on the same plant, and that the cause of self-sterility after an incompatible pollination is due to the failure of pollen tubes to grow rapidly enough to reach the ovary before the flower falls. Martin [1913] working with *Trifolium pratense*, a self-sterile form, found that the difference between the self and cross pollinations was of the rate of growth of the pollen tube. East and Park [1917] in their studies on *Nicotiana*, have secured abundant evidence to show that following incompatible combinations, the rate of growth of pollen tube in the style is lower than that attending fertile combinations.

Very little work has been done on the sterility of Brassicas. Price [1912] discussing a cabbage hybrid says "Attempts to self-pollinate individual flowers resulted in failure. It was also found practically impossible to secure seed by crossing different flowers on the same plant ".

Howard, Howard and Khan [1910] conducted preliminary investigations in *sarson*, *rai* and *toria*. These authors showed that *sarson* readily sets seed under bag, and *toria* do not set so easily as *sarson* and *rai*. Ali Mohammad, Singh, and Alam [1939] have also shown recently, that *toria* and black *sarson* are highly self-sterile forms, and that loss of vigour results on inbreeding.

III. Presentation of data.

1. The determination of the degree of self-sterility.

Single plant cultures were grown in 1930-31 from the seeds harvested in the previous year. These cultures were grouped into nine different morphological groups. The amount of seed setting and the percentage of setting worked therefrom is given in Table I. The percentage of setting under bag is usually less than that in the open condition owing to the high humidity prevailing inside the bag. Manilla paper bags and muslim cages were employed for bagging the plants. The setting under these bags takes place freely in the self-fertile brassicas grown at Pusa. For working out the percentage of setting under bags it is assumed that all flowers bagged would form healthy pods. It was observed that some flowers on the unbagged flower branches did not produce any pod. Thus the percentage of setting calculated is represented to be somewhat less than what it ought to be. For calculating the amount of setting in a group the actual number of seeds that are formed are taken into consideration, and the number of pods that are formed are not taken into account. It has been observed that the pods, which succeed in setting

inside bags contain only a fraction of the number of seeds that are usually contained in pods on free flowering plants. Empty pods have also been recorded in many instances.

TABLE I.

Showing the setting of seeds under bag in the different brassica groups—1930-31.

	Name of group	No. of plants bagged	No. of flowers bagged	No. of pods set	No. of seeds formed in the bagged pods	Average no. of seeds per pod outside the bag	No. of seeds expected in the normal pods*	Per-centage of setting
1	Yellow *sarson* . .	77	2054	1843	31243	24	49296	63·4
2	Black *sarson* late . .	64	4174	756	3609	16	66784	5·4
3	*Toria*	274	6631	296	558	13	86203	0·3
4	Black *sarson* early . .	99	4189	592	2501	16	64262	3·9
5	Burma *rai* . . .	31	2232	510	4712	21	46872	10·5
6	White *rai* . . .	7	269	0	0	10	0	0·0
7	Asl *rai*	18	4546	2023	3567	7	31822	11·2
8	*Rai* ordinary . .	148	5377	4249	39112	15	80655	48·5
9	*Rai* (young habit cabbage like)	54	6290	4285	34293	15	94350	36·3

* Number of seeds expected is worked out by multiplying number of flowers bagged × average number of seeds per pod outside the bag.

The percentage of setting is 63·4 in yellow *sarson*, 48·5 in *rai* ordinary, and 36·5 in *rai* (young habit cabbage like). Other groups have very low percentage of setting and are regarded as self-sterile groups.

Large number of *taramira* plants were also bagged, but the setting was almost negligible.

2. *The cause of self-sterility.*

Self-sterility may be due to a number of causes either acting singly or jointly. Only those factors which effect the sterility in brassicas are dealt here.

(*a*) *Environmental factors.*—The most important of these is the cloudy weather. In self-sterile varieties of brassica the seed is set only by cross-pollination brought about by bees, which visit the flowers in large numbers on sunny days. On cloudy days the bees do not visit the flowers, and this fact may perhaps adversely affect normal setting in these varieties. The effect of cloudy weather is uniform for all the

brassica groups, but self-fertile varieties are not affected. They can form seed with their own pollen. This is a very important factor for self-sterile varieties. If there happens to be a long period of cloudy weather, the yield is badly affected.

(*b*) *Behaviour of gametes.*—Sterility may also be due to defective gametes. So far the pollen has not been found to be defective in the brassicas under investigation.

The self-fertile varieties freely form seed with their own pollen. On the other hand, the self-sterile varieties do not set seed under bag, or very rarely they do so. It is also observed, that when flowers are selfed in the bud stage (one to two days before opening), pollen from the same plant being employed, the self-sterile varieties could be made to set a good amount of seed. These self-sterile varieties also form good seed when flowers are crossed, pollen from different plants being employed. In cross-pollinations (compatible matings) the amount of setting is much higher than in self-pollinations (incompatible matings). The results are tabulated below.

TABLE II.

Showing difference in amount of setting in cross and self-pollinations in taramira 1929-30.

No. of plant	Selfed just after the flowers opened with pollen from the same branch.		Selfed just after the flowers opened with pollen from different branches		Selfed in the bud stage with pollen from different branches		Crossed with pollen from different plants	
	Flowers selfed	Pods formed	Flowers selfed	Pods formed	Buds selfed	Pods formed	Flowers crossed	Pods formed
1	11	0	11	0	13	6	8	8
2	8	0	7	1	12	4	6	6
3	9	2	7	6	11	7	7	7
Total .	28	2	25	7	36	17	21	21
Percentage setting .	7·1		28		47·2		100	

The defect in the female organ (ovules) has only been recorded in *taramira*. It is a cross-fertilized crop and is completely self-sterile. No two plants in a single plant culture are alike. It is observed that some plants in a culture bear large number of fruits, while there are other plants which either do not bear any fruit, or the fruits are very poor without any seed. The chances for the bees to visit the

flowers in all cases being the same, we may say that the plants which do not form fruits in the unbagged branches are defective in the female organ (ovules).

This fact is also confirmed by the following experiment which was made to see the difference in fertility of the progenies between two similar plants, when each is selfed in the bud stage, and each is crossed with the other. However, incidentally it has been shown that sterility is sometimes due to some defect in the female organ.

Four pairs of *taramira* plants were selected, the plants of each pair being similar in all morphological characters as far as possible. In each pair the plants were designated A and A′ and the following operations were conducted.

The plants A and A′ were selfed in the bud condition with their own pollen. Plant A was crossed with A′ and the reciprocal cross A′ × A was also made. The results are tabulated below.

TABLE III.

Showing percentage of setting in four pairs of taramira *plants, when both the plants of a pair are selfed with their own pollen and each is crossed with the pollen of the other—1930-31.*

Pair No.	Selfing in the bud			Crossing in the bud			Remarks
	Operation No.	No. of buds operated	No. of pods formed	Operation No.	No. of buds operated	No. of pods formed	
1	A	40	0	A × A′	31	0	♀ defective
	A′	30	5	A′ × A	30	14	
2	A	40	10	A × A′	30	15	
	A′	40	0	A′ × A	40	0	♀ defective
3	A	25	0	A × A′	20	0	♀ defective
	A′	30	6	A′ × A	40	17	
4	A	40	34	A × A′	40	29	
	A′	42	16	A′ × A	40	19	

The plants A in pairs Nos. 1 and 3 have not formed pods in both the operations, *i. e.*, when A is selfed, and also when A is crossed with A′. In pair No. 2, the plant A′ has behaved in a similar manner. In pair No. 4, pods have formed in all the four operations. The percentage of pod formation is distinctly lower in A′ both when selfed and cross-pollinated. This suggests partial defect in the female A′. The plants A′ in pairs Nos. 1 and 3, and plant A in pair No. 2, have formed pods in both the operations. If the pollen had been defective the setting would not have taken place in both the plants A and A′ in the pairs Nos. 1, 2 and 3, as the same pollen was used for plants A and A′.

For selfing and crossing the plants of a pair, pollen was collected from plants A and A′ in separate sterilised dishes. The plant A was selfed with its own pollen, and the same lot of pollen was also used for crossing plant A′ of the same pair. Similarly plant A′ was selfed with its own pollen and the same lot of pollen was also used for crossing plant A of the same pair.

Very likely in *taramira* when the pods are not formed the defect is in the female organ. This fact has not been recorded in other brassicas.

It is also clear from the above table, that the percentage of pod setting is generally higher when the buds are crossed.

3. Selective pollen tube growth.

To study the rate of growth of the pollen tube in compatible and in incompatible unions a series of self-pollinations and cross-pollinations were made as detailed below. Pistils were collected at different intervals of time after pollination, fixed, dehydrated, embedded, and sectioned in the usual manner. Longitudinal sections 10 μ thick were cut and stained with Diamont fuchsin single stain.

The culture selected for this purpose was No. 142-5 black *sarson* early, which was found to be completely self-sterile.

The experiment was started on the 17th January 1931 and the operations A, B, C and D were conducted at the same time (9-30 A. M.).

Operation A.

A_1 :—Twenty-five pistils taken from buds (one to two days before opening) and fixed.

A_2 :—Twenty-five pistils were fixed (from flowers at the time of opening).

Operation B.

Fifty flowers which opened under muslin bags were selfed; pollen from the same flowers being used, and the plant was again bagged.

Operation C.

Fifty buds (one to two days before opening) were selfed with the pollen from different flowers on the same plant. The plant was again bagged.

Operation D.

Fifty flowers which opened under bag were crossed with the pollen of the other bagged plant. The plant was again bagged.

In operations B, C, and D only those flowers which were operated in each case were kept on the plant, and the remaining flowers were removed. These operated flowers were also labelled.

Out of fifty flowers operated in each of the B, C, and D operations, twenty-five were fixed in each case after different intervals, and the remaining twenty-five were allowed to remain on the plant to see the amount of setting in each case.

TABLE IV.

Showing amount of setting in operations B, C, and D.

Operation	No. of operated flowers or buds kept on the plant	No. of pods formed	Total number of seeds formed
B	25	5	5
C	25	24	119
D	25	17	78

The highest amount of seed formation is in operation C. In operation D the amount of seed formation in only $\frac{1}{5}$ of operation C. In operation B the amount of setting is negligible.

Crossing seems to give more stimulus to fruit formation than does selfing. The pods formed in operation D are healthy in appearance and are very much like the pods formed by the free flowering branches. In operation C the pods formed are not as large as those formed in operation D. The pods formed in operation B are very poor.

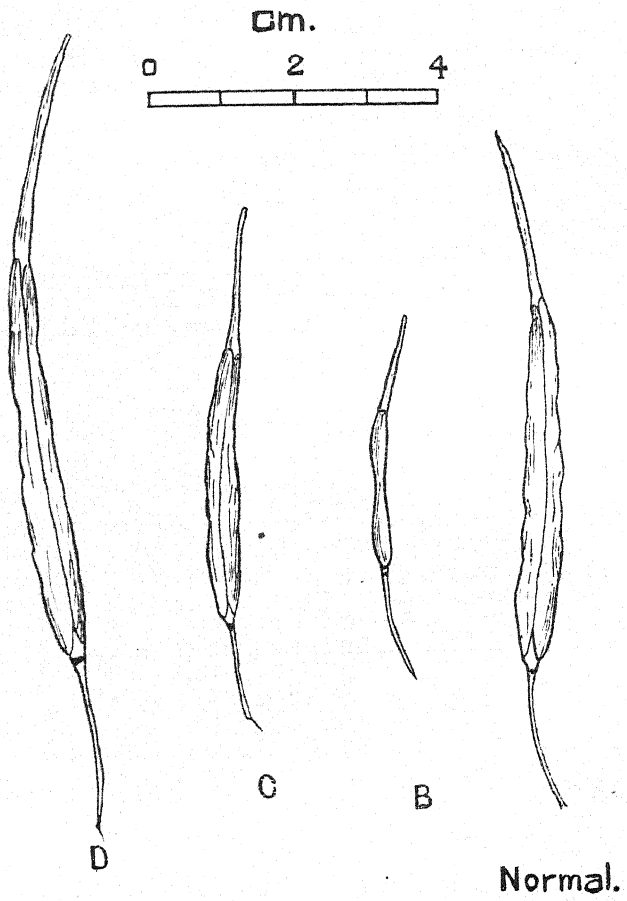


Fig. 1.—The largest pods formed in operations B, C and D, compared with the normal pod on the free flowering branches.

Pollen tube growth :—The pollen tube after passing through the papillate cells of the stigma proceeds along the conducting strands of the style and the wall of the ovary. The pollen tubes shown in the figure are reconstructed from serial sections. The time taken by the pollen tube to reach the uppermost ovule is determined from the sections prepared from the material, which was fixed after different intervals of time after pollination.

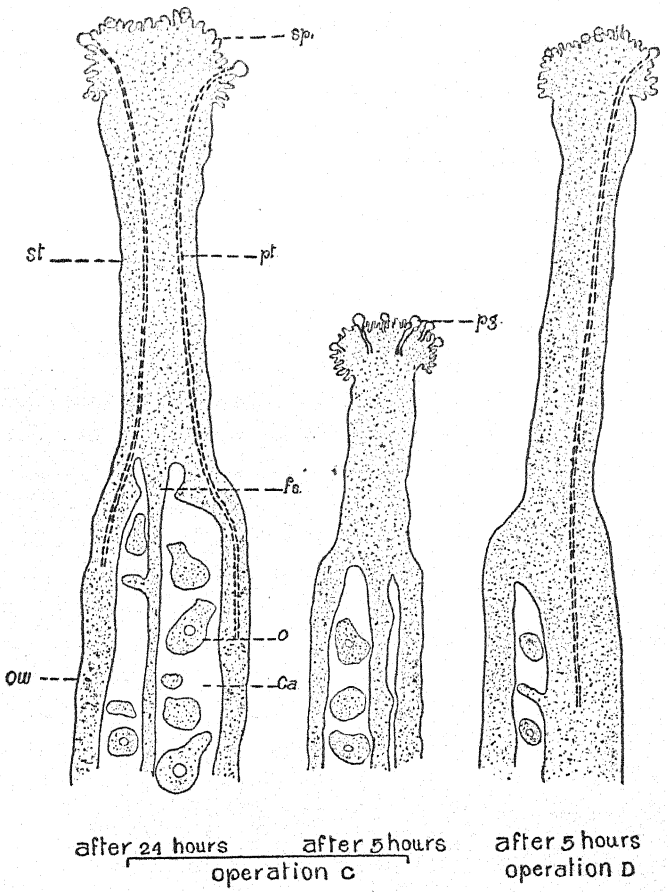


Fig. 2.—Longitudinal sections—Pistil (diagrammatic and reconstructed), showing the rate of pollen tube growth. *Pollen tube is shown by a double dotted line.* ca, cavity of the ovary. fs, false septum. o, ovule. ow, wall of the ovary. pg, pollen grain germinating on the stigma. pt, pollen tube. sp, papillate cells of the stigma. st, style.

TABLE V.

Showing rate of growth of the pollen tube in the operations B, C, and D.

Operations	Details of the operations	Time taken by the pollen tube to reach the ovary after pollination
B	Flowers selfed in the open condition . .	48 hours
C	Buds (1-2 days before opening) pollinated. Pollen from different branches of the same plant used.	24 hours
D	Flowers crossed with pollen (of different plants)	5 hours

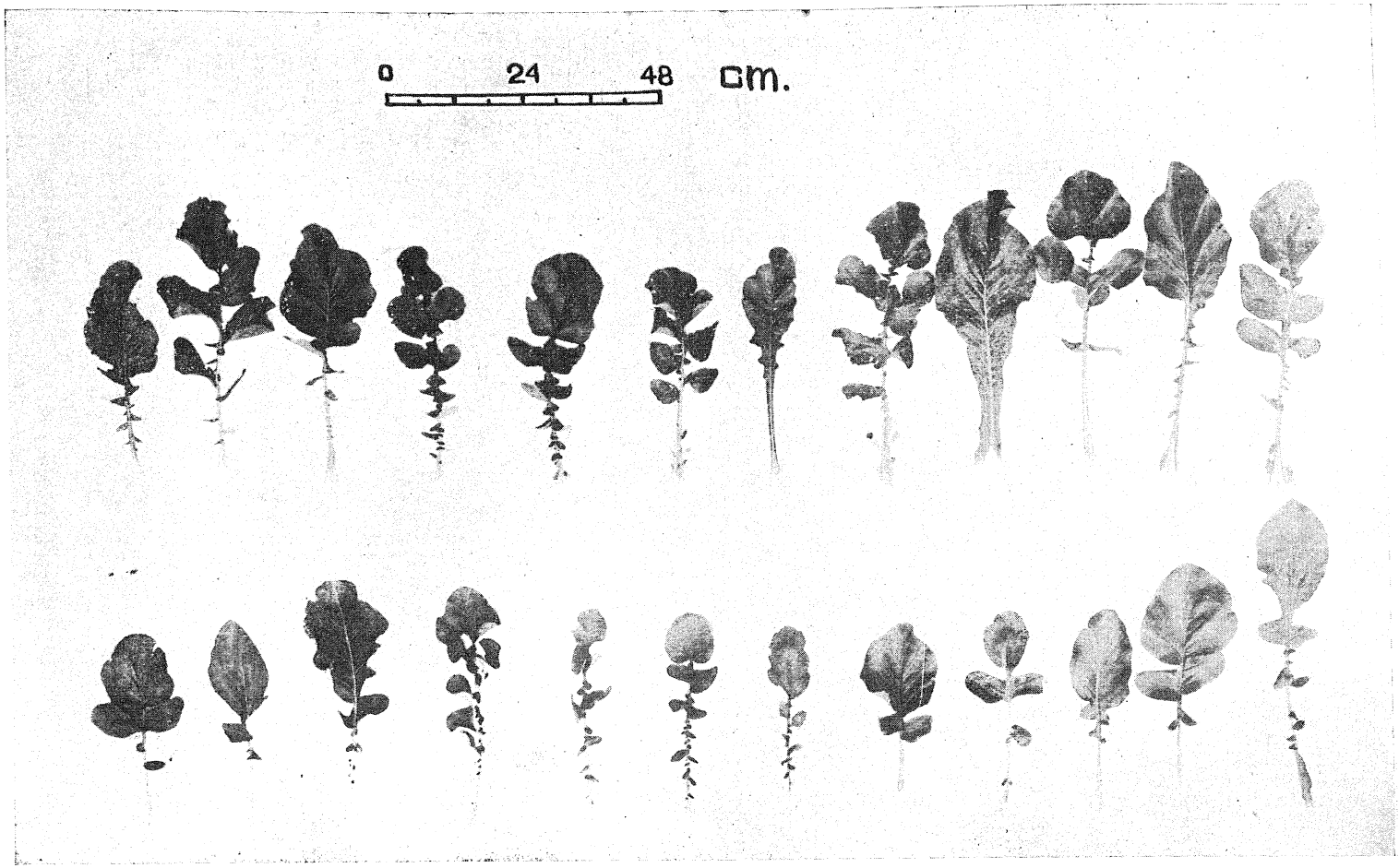
The above table shows that the rate of the pollen tube growth has much to do with the amount of seed formation and the degree of sterility. Examination of the slides from the material A_1 and A_2 show, that there is not much difference between the lengths of the styles of open flowers and buds (1-2 days before opening), and that the pollen tube has to traverse the same length in all cases.

The highest amount of seed formation is in operation C, in which buds (1-2 days before opening) were selfed. In this operation the pollen tubes have taken twenty-four hours to reach the ovary after pollination. These buds opened 1-2 days after pollination. It means that the pollen tube reached the ovary even before the flower actually opened. In operation D in which the flowers were crossed with pollen of different plants, the amount of setting is also quite high. The time taken by the pollen tubes to reach the ovary is five hours after the opening of the flowers. The least amount of setting is in operation B when the flowers were selfed. The pollen tube has taken forty-eight hours to reach the ovary and the amount of setting is almost negligible. The rate of growth of the pollen tube is quicker in operation D (when flowers are crossed) than in other operations but the least time taken by the pollen tube to reach the ovary after the opening of the flower is in the operation C (when buds 1-2 days before opening are selfed).

The pollen germinates in operations B, C and D, and the pollen tube reaches the ovary in almost all cases. It is the time which the pollen tube takes in different cases, which accounts for the amount of setting. If the tube reaches at the right time, there is a good amount of setting, otherwise either the setting is very poor or there is no setting at all.

IV.—DISCUSSION.

Referring first to self-incompatiblity it seems quite clear, that the cause of incompatibility is the well-known inability of the pollen tubes to proceed through the stylar tissue. That is to say, the gametes generally are quite capable of uniting successfully, but they never meet at the right time. The ovule is receptive even one day before the flower opens. In self-sterile black *sarson* early group when the



The variation in leaf form, size and shape in a single plant culture of *black sarson late* (*Brassica campestris*. L. var. *oleifera* Prain).

buds are selfed (1-2 days before opening), the pollen tubes take twenty-four hours to reach the ovary, that is to say, fertilization takes place actually before the flower opens. When cross-pollination is made the growth of the pollen tube is very quick, and the union of the gametes takes place before the ovule loses its receptive power. In self-pollinations the growth of the pollen tube is very slow, and only in rare cases it may meet the ovule at the right time.

The inability of the pollen tubes to proceed through the stylar tissue in incompatible matings is generally attributed to the production of inhibiting substances or to the non-production of some stimulating substances.

Darwin [1876] made a thorough investigation on the effects of self-fertilization on the offspring, and showed that in flowering plants such offsprings are not in general so vigorous as those derived from cross-pollinations. This phenomenon has been observed in brassicas.

To explain the results of compatible and incompatible matings Jost [1907] had recourse to the old conception of "Individualstoffe". He believes that individuals not only of the same species, but of the same type differ qualitatively in their chemical composition. The gametes of any plant possess the "Individualstoffe" of that plant, and that the pollen tube grows well only in tissues having a different "Individualstoffe".

East and Mangelsdorf [1926] working on *Nicotiana* have shown that the sterility is determined by the allelomorphic factors S_1, S_2 and S_3. The action of these sterility factors is such that the growth of the pollen tubes carrying a given factor is inhibited in the style of the plant carrying that factor.

Whatever the explanation be, it is evident that the pollen tubes grow more quickly in compatible unions than in incompatible matings.

In black *sarson* late which is a completely self-sterile group, there is so much cross-pollination going on that no two plants have similar leaves in a single plant unbagged culture (Plate XXIII). In self-sterile brassicas the offspring of self-fertilized seed show very poor growth, but the self-fertile brassicas do not show any loss of vigour on self-fertilization.

The vigour, fertility and the very existance of these sterile brassicas is due to cross-pollination.

V. Acknowledgments.

The writer wishes to acknowledge his indebtedness to Dr. F. J. F. Shaw, Imperial Economic Botanist, Pusa, and Khan Sahib Abdur Rahman Khan, Superintendent, Botanical Sub-Station, Karnal, for their helpful criticism and advice.

The results tabulated in Table I were done jointly with S. Hukum Singh and M. Habibur Rahman Khan of the Botany Department, Pusa, to whom my thanks are due.

VI. Summary.

1. The brassicas under investigation at the Botanical Section, Pusa, comprise nine distinct morphological groups, yellow *sarson*, *rai* ordinary and *rai* (young habit cabbage like) are self-fertile; and black *sarson* late, *toria*, black *sarson* early, Burma *rai*, white *rai*, *asl rai* and *taramira* are self-sterile.

2. The pollen has not been found to be defective in the brassicas under investigation, but in *taramira* it has been observed that the sterility may be due to the female organ being defective.

3. Self-fertile varieties freely form seed with their own pollen. On the other hand, self-sterile forms do not set seed with their own pollen. In self-sterile varieties the amount of setting in cross-pollinations is much higher than in self-pollinations.

4. In self-sterile varieties the cross-pollination is effected by bees, which visit the flowers in large number on sunny days. If there happens to be a long period of cloudy weather the yield is badly affected.

5. It is shown that in compatible unions the pollen tube reaches the ovary in a shorter time than in incompatible matings, and the sterility in incompatible matings is due to the failure of the pollen tube to reach the ovule before it has lost its receptive power.

6. In self-fertile varieties the offsprings of a self-fertilized seed are vigorous and fertile, but in self-sterile varieties the offsprings of self-fertilized seed show very poor growth.

7. The self-sterile varieties are adapted to cross-fertilization, and it is probably due to cross-fertilization that the vigour and the fertility of the sterile groups are maintained.

VII.—References.

Ali Mohammad, Singh and Alam (1930). *Ind. J. Agric. Sci.* **1**, 109—136.
Compton (1913). *New Phytologist* **12**, 197—206.
Correns (1912). Summary in East and Park, *Genetics* **3**.
Darwin, C. (1876). The effect of cross and self-fertilization.
East and Park (1917). *Genetics* **2**, 505—609.
East and Mangelsdorf (1926). *Genetics* **11**, 466—481.
Howard, Howard and Khan (1910). *Mem. Dept. Agric. Ind. Bot. Ser.* **7**.
Jost (1907). *Bot. Ztg.* **65**, 77—117.
Knuth (1906). Handbook of flower pollination Vol. **1**, 360.
Loew (1895). Einführung in die Blütenbiologie auf historischer Grundlage, Berlin, pp. 432.
Martin (1913). *Bot. Gaz.* **56**, 112—126.
Prain (1898). *Agric. Ledger* **5**, 1—78.
Price (1912). *An. Rep. Virginia Polytec. Inst. Agric. Exp. Stn.*
Scott (1865). *J. Lin. Soc. London* **8**, 163—167.
Stout (1917). *Amer. J. Bot.* **4**, 375—395.
—— (1922). *Bot. Gaz.* **73**, 111—132.

A STUDY OF THE PATHOLOGICAL ANATOMY OF THE COTTON PLANT IN CONNECTION WITH THE WILT DISEASE.

BY

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(Received for publication on the 18th November 1931)

(With plates XXIV & XXV and four text-figs.)

INTRODUCTION.

With the discovery of the phenomena of susceptibility and resistance to disease among plants, a new era in the method of control of plant diseases had commenced. Breeding resistant types through selection or hybridization is the only effective means of checking those diseases like flax wilt, cabbage yellows, and cotton wilt caused by species of *Fusarium*, which inhabit the soil.

The need for information on the nature and cause of resistance had become increasingly evident, with the development of varieties resistant to disease. Intensive research on the problem of disease resistance is being carried on from the last one or two decades. The diseases, root rot of tobacco, flax wilt, yellows of cabbage, root rot of cotton and rusts of wheat have been fully investigated with a view to find out the behaviour of the fungus towards types that vary in the degree of resistance and also to learn the nature of response of the host cells to fungus penetration.

There was no record, however, of any study on the nature of resistance to wilt in cotton or the mode of infection of *Fusarium vasinfectum*, Atk., in the susceptible, resistant, and immune varieties of cotton, when the present study was undertaken in April 1928.

Fahmy [1928] observed that wound infection in a susceptible variety of cotton was able to produce the typical discoloration in the central cylinder at the injected portion, while in the immune variety it was not developed.

In a later publication [1930]* Fahmy described the mode of peneration of *Fusarium vasinfectum*, Atk. var *aegyptiacum* in cotton. He observed the parasite attacking the seedlings through the roots, developing first of all an almost black discoloration on the outer surface of the lower part and a brown one on the upper part. With the progress of the disease the rootlets as well as the greater part of the radicle disintegrated. The root attacked by the fungus rotted at first in the

*This paper came to the notice of the writer in December 1930, after the present study was completed.

region of the root-cap and later on the parts above it completely disintegrated. Fahmy thought that it was the disintegration of the young roots which caused the death of the seedlings.

As regards penetration of the fungus, he found that it first attacked the root-cap, though its later activity was not confined to the invasion of the root-cap alone. It was found also to penetrate and invade the tissues of the root proper. The hyphae got accumulated on the thin parts of the root-cap as the fungus was pushed back in its repeated attacks on it. Later on it transformed the cell inside, which formed, with the neighbouring cells similarly invaded, a thick mass of filaments replacing the cellular structure of that part of the root. The progress of the fungus in the tissues beyond this strongly invaded region, was along the inter-cellular spaces and not through the interior of the cells.

Relation of soil temperature to cotton wilt.

During the course of the cotton wilt investigation in the Cotton Research Laboratory, Dharwar, the close relation between the soil temperature and the incidence of the wilt disease of cotton had been observed by Kulkarni.* It has been noted that the desease was at its optimum between temperatures 18°-25°C. Even the resistant varieties were found to show a great degree of susceptibility at the above temperatures. As the temperature rose from 25°C. onwards to 30°C. there was a decrease in the disease while it was eliminated at 32°C. and above. This remarkable response of the disease to soil temperature presented a fine opportunity for the study of the nature of resistance to wilt in cotton.

The work recorded in this paper was undertaken with a view to find out :—

1. The mode of penetration and the further progress of *Fusarium vasinfectum* under ordinary atmospheric conditions, in three varieties of cotton plants (susceptible, resistant, and immune) that vary in the degree of their resistance.

2. Whether there are any histological differences that distinguish the three varieties.

3. How the three different hosts react toward the infecting hyphae under controlled conditions of temperature.

Material.

Three varities of cotton that markedly differed in the degree of their resistance to wilt were chosen for this study.

*Unpublished records referred to with permission.

Dharwar I cotton (*Gossypium herbaceum*, L.)—a selection from the Kumptas—whose wilt percentage is very high, was selected as the typical susceptible type. Dharwar II cotton (*Gossypium herbaceum*, L.)—also a selection from the Kumptas—which resists to the extent of 96 percent in the fields was chosen for its resistance; while Gadag I (*Gossypium hirsutum*, Mill.) a strain from the acclimatized American cotton was selected for its immunity.

A pure culture of *Fusarium vasinfectum* which was isolated from wilted cotton plants of Dharwar I variety in September, 1924 and whose parasitism has been tested, was used throughout the present study.

Methods.

Four to five delinted* selfed seeds of Dharwar I, Dharwar II, and Gadag I, were sown in sterilized Petri dishes, the bottoms of which were covered with moist blotting papers. The radicles generally emerged on the second or third day. When they were sufficiently big, *i. e.*, about an inch or an inch and a half long, a definite portion behind the root-cap, about half to one centimetre, which had very often root hairs, was inoculated with a suspension of spores, in sterile water obtained from a ten days old culture of the cotton wilt fungus, grown on steamed rice. A microscopic examination of the inoculum showed a large number of microspores, a few macrospores and a few hyphae. The inoculated portion was marked off with Indian ink. Care was taken to see that the seedlings were not disturbed from their original places. The Petri dishes were kept in the plant-house, the temperature of which ranged from 12°-32°C. during the period of investigation.

The inoculated portions of the roots were cut and fixed in weak chromacetic acid from the first day to the eighth day. The material was washed and dehydrated by the usual methods, embedded in paraffin (53°C. M.P.) and sections were cut 10μ or 12μ thick. Safranin and gentian violet combination was used.

For the study of the effect of soil temperature on the wilt, plants of the three different varieties mentioned above, were grown in sterilized soil—soil* sterilized at 15 pounds pressure and 135°C. for an hour. For the low temperature series the infected pots as well as their controls were kept in big earthenware pots containing cold water. For the sake of convenience they are referred to in the following pages as cold tanks. The temperature of these cold tanks never went above 25°C. For the higher ranges of temperature, the special incubators in the Cotton Research Laboratory, which could maintain the required temperature were made use of. The plants were grown in infected soil in the incubators at 32-34°C. The study of the pathological anatomy of these plants grown at high and low temperatures, was made by hand sectioning.

*The seeds were delinted by treating them with concentrated sulphuric acid for five minutes and they were then thoroughly washed with distilled water.

The nature of substances produced in response to the fungus attack was found by treating the hand sections of the roots from infected and sterile soil, with iodine, chlorzinc-iodide, potassium hydroxide, Schulze's macerating fluid and sudan III. Schulze's macerating fluid was used throughout the present study to test for suberin.

INVESTIGATIONS.

Anatomy of the normal root.

In order to see whether there are any anatomical peculiarities that distinguish the roots of the susceptible, resistant, and immune varieties selected, sections of the roots of Dharwar I, Dharwar II, and Gadag I were examined microscopically. But no such differences could be observed. The anatomy of the normal root of cotton is, however, described for a clear understanding of the following pages.

The primary structure of the cotton root resembles that of any typical dicotyledonous root. It consists of uniseriate epidermis which develops root hairs some distance behind the elongating region. The cortex consists of six to seven and sometimes even eight layers of thin walled parenchyma.

The stele is surrounded by the pericycle consisting of a layer of parenchymatous cells and a prominent endodermis. The four protoxylem bundles alternate with an equal number of protophloem bundles. The central part of the stele is occupied by the pith.

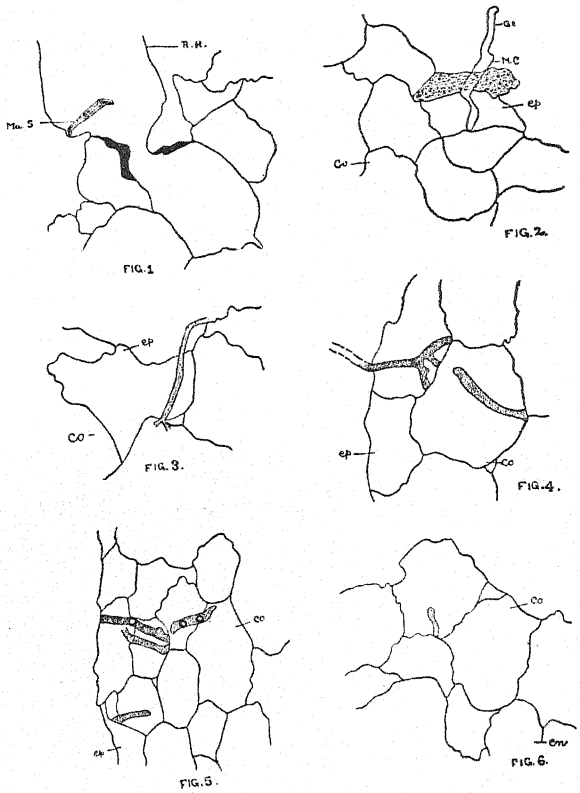
The secondary thickening commences during the fifth week after germination. The epidermis, cortex and the endodermis completely disappear only in the sixth week after germination and it is at this time that the cork cambium (Phellogen) is formed and cork is produced externally.

A. Penetration and progress of the cotton wilt fungus in the susceptible variety.

The micro and macrospores of *Fusarium vasinfectum* are hyaline. The microspores are of varying shapes. They are unicellular as well as septate. The macrospores are sickle shaped and are more often septate. Ordinarily under normal conditions both the micro and macrospores germinate within twenty-four hours. But on the radicles the germination was delayed till the second day after inoculation. In some cases they were found to remain on the radicles without germinating till the fifth or the sixth day. Small strips of epidermis were peeled off at intervals and examined in water under the microscope. The ungerminated spores used to float in water.

Butler [1918 ..] is of opinion that " moisture is probably the most important condition which controls the germination of spores". The delay to germinate more readily on the radicles might be due to many causes, of which the want of

sufficient moisture might be one. The germ tubes were put forth from one or both ends of the spore. In fig. 1, a macrospore is seen attached to a root hair. Small pieces of hyphae that were carried along with the spores were also seen attached to the outer wall of the epidermis.



Stages of penetration and progress of the cotton wilt fungus in Dharwar I.

Fig. 1. Two days after inoculation. A macrospore is seen attached to the root hair. Ma. S.—Macrospore; R. H.—Root hair. ×768.

Fig. 2. Four days after inoculation. A microspore (M. C.) has germinated and the germ tube has made its way into the epidermis (ep). Co.—Cortex. Gt.—Germ tube ×390.

Fig. 3. Five days after inoculation. The fungus has entered the cortex. ep.—epidermis.; Co.—Cortex. ×128.

Figs. 4 and 5. Six days after inoculation. The rapidity of the fungus penetration and its progress in the cortex can be seen. ×390.

Fig. 6. Seven days after inoculation. The fungus has reached the last layer of the Cortex. ×390.

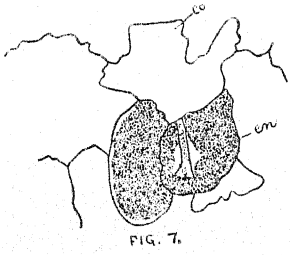


FIG. 7.

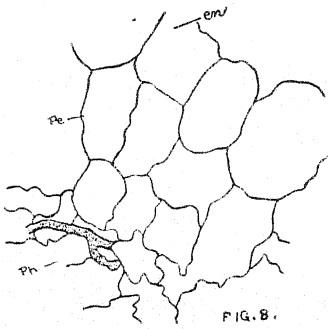


FIG. 8.

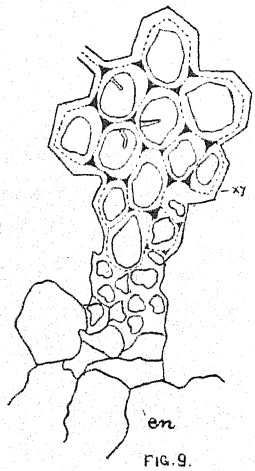


FIG. 9.

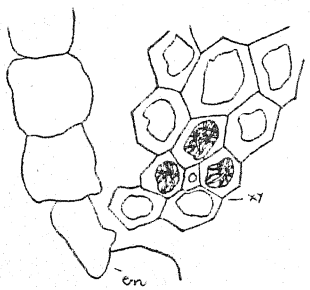


FIG. 10.

Stages of penetration and progress of the cotton wilt fungus in Dharwar I.

Fig. 7. Eight days after inoculation. The travelling of the fungus through the endodermis (en) is seen. Co.—Cortex. ×128.

Fig. 8. Eight days after inoculation. A hypha is seen in the phloem (ph). Pe.—Pericycle.; en.—Endodermis. ×128.

Fig. 9. Eight days after inoculation. Hyphae are seen penetrating into the xylem (xy) vessels. en.—Endodermis. ×768.

Fig. 10. Eight days after inoculation. Hyphae are seen clogging the xylem (xy) vessels. ×128.

The germ tubes penetrated the epidermis either directly without elongating on the surface of the radicle or they elongated and branched before they penetrated. By the third day after inoculation, a number of hyphae could be seen spreading

over the inoculated portion of the radicle. But not a single case had been observed, in which entrance was gained by the hyphae during this day.

At the time when the fungus was attempting to enter the root there were no secondary roots developed. The fungus attacked the tap root first. The infected portion of the root occasionally showed slight yellowing by the third or the fourth day. Hand sections of fresh material showed the outer walls of the epidermis coloured very slightly yellow. The germ tubes or the hyphae were seen to penetrate the root through the outer wall of the epidermis, on the fourth day after inoculation. The hyphæ were never found to enter the host tissues through the root hairs, though there were a number of them in the neighbourhood of the cells penetrated. The hyphae were seen to make their way singly into the host through the outer wall of the epidermal cell. The epidermal cell into which the hypha penetrated showed no visible signs of any disturbance in its contents, either before or after the entry of the fungus. There was neither any constriction of the hypha at the point of its entrance, nor did the cell wall surrounding the hypha appear thin or weakened. Where the hyphae entered, there was not more than one hypha making its way in through the same point. Hyphae were not found to " surge through " the cell wall. The hyphae after having entered the epidermis showed no tendency either to swell or shrivel. Fig. 2 shows a typical stage of fungus entry. The germ tube has entered and pushed itself into the cell to a certain extent. These observations point to the probability that the entry of *Fusarium vasinfectum* into the root of cotton is purely mechanical.

The fungus travels through the epidermal cells and pierces through the inner wall of the epidermis to enter the cortex on the fifth day after inoculation. The penetration is effected in the same manner as before. No discoloration or plasmolysis takes place in the cortical cells beneath the point of attack. Though sometimes the hyphae are found to go very close to the cell wall, their general habit in travelling from one layer to the other is by going right across the cell from the outer wall to the inner one. They are not observed to progress in the tissues by making their way between the cell walls. The method of entry as well as the progress of the fungus is intracellular and not intercellular. Branching of the hyphae in the tissues is far from being common.

The progress of the fungus, after it entered the cortex is very rapid. It has gone half way into the cortex after the sixth day after inoculation. The hyphae are turgid all along. They do not appear to be injured, which shows that the host tissues have not reacted adversely towards the progressing hyphae. The fungus does not show any tendency to clog the epidermal or cortical cells, either by their accumulation or by their free branching in those cells. The ease and quickness with which the cortical cells are invaded by the fungus and the absence of any

disorganization of the contents of these cells, suggest that no resistance is offered to the progressing hyphae by the invaded tissue. The only barriers in the way of the fungus are the cell walls.

By the sixth day after inoculation the fungus has completely surrounded the outer surface of the root, and fresh entries of the hyphae are observed up to this day.

On the seventh day (after inoculation) the fungus travels through the remaining layers of the cortex as before and reaches the endodermis. No other changes are observed on this day.

The endodermis and the pericycle are crossed the next day (eighth day of inoculation) and the hyphae enter the protoxylem vessels also. The endodermal cells are turgid with protoplasmic contents and are darkly stained. There are cases (Fig. 7) where the fungus is making its way through the endodermis. The fungus is also seen travelling through the phloem region (Fig. 8). The free ends of the hyphae that penetrate through the walls of the xylem are seen in the vessels (Fig. 9). In some of the advanced cases the interlacing of the filaments also can be found in the vessels. The entry into the protoxylem vessels is made possible in such a short time by the nearness of those vessels to the endodermis. (Figs. 9 and 10.) The phloem vessels are not clogged by it. The typical discoloration that is seen in the wilted plants could not be clearly seen in the stained slides. In fresh hand sections of the root of the same age, yellowing of vessels and a very slight brown discoloration in some cases were observed.

No other histological changes in the cortical and endodermal tissues were found. *Fusarium vasinfectum* is a vascular fungus and it enters the susceptible host, Dharwar I, with the least resistance, travels through the epidermis, cortex, endodermis and pericycle without affecting them visibly and reaches the xylem vessels, which it inhabits thereafter.

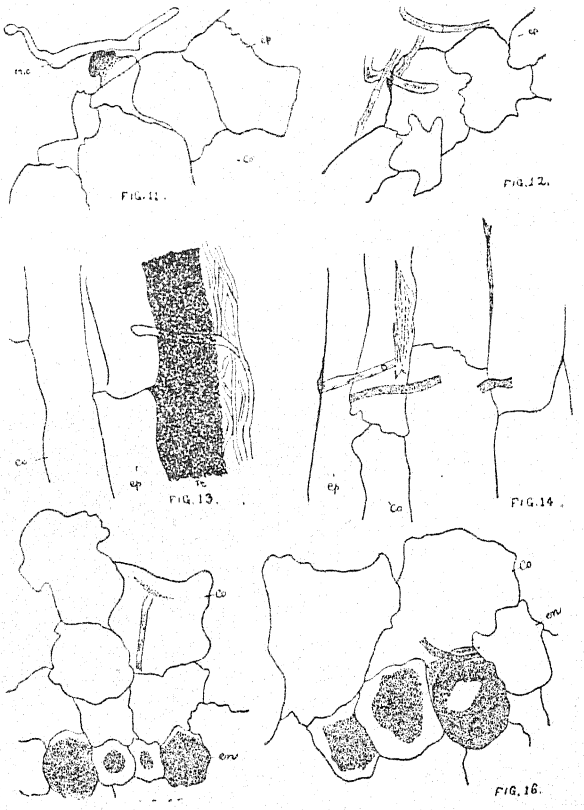
B. Penetration and progress of the cotton wilt fungus in the resistant and the immune types.

The fungus penetration and the manner of its progress in the resistant and immune types were so similar that they are here described together.

The germination of the spores was generally on the second day after inoculation in both the types, though in one or two cases they were observed to put forth germ tubes even on the first day. Absence of favourable environmental conditions seems to be the only cause of retardation of the germination of spores.

Though the germ tubes emerged on the first or second day after inoculation, the penetration into the epidermis could not be effected till the fourth day as in the susceptible variety. The germ tubes and the hyphae, that were carried along with the spores, grew and spread over the outer surface of the root. A layer of cork, varying in its thickness, was formed over the epidermis of the infected portion of

the root (Figs. 18 & 19). The uninfected portion showed no such development. Even the infected portion did not show a cork layer of uniform thickness. It was thicker where the hyphae were trying to enter into the tissues. The outer walls of the epidermal cells were also suberized. The presence of suberin was tested by Schulze's macerating fluid. The resistant and immune varieties had developed the peripheral cork and suberin in the outer epidermal walls, unlike the susceptible variety, in response to the attempt of the fungus to enter the tissues.



Stages of penetration and progress of the cotton wilt fungus in Dharwar II.

Fig. 11. One day after inoculation. The germination of the microspore (M.C.) on the epidermis (ep) of Dharwar II. Co.—Cortex. ×128.

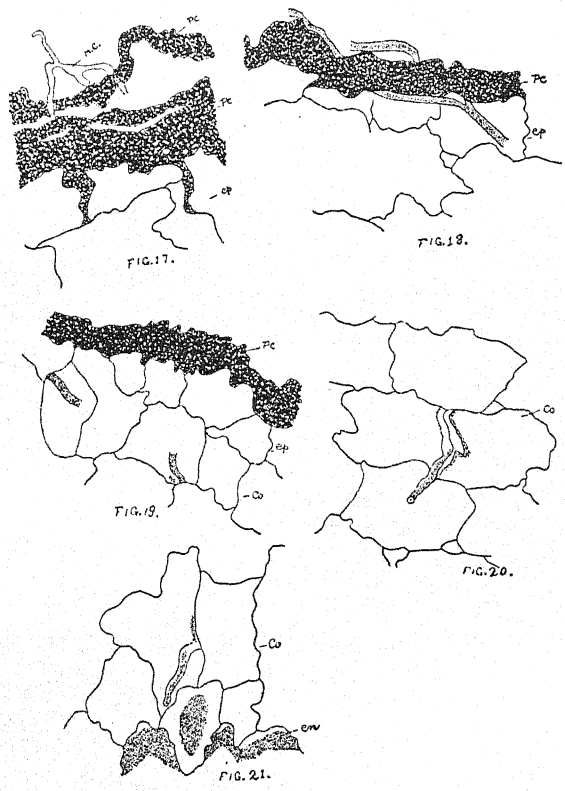
Fig. 12. Five days after inoculation. The hypha has entered the epidermis (ep). ×390.

Fig. 13. Four days after inoculation. The hypha is penetrating into the epidermis, after passing through the peripheral cork. (Pc.) ×390.

Fig. 14. Six days after inoculation. The progress of the fungus in the cortex can be seen. (Longitudinal section). ×390.

Fig. 15. Seven days after inoculation. The fungus has reached the last but one layer of the cortex. ×390.

Fig. 16. Eight days after inoculation. The entry of the fungus into the endodermis (en) is seen. Co.—Cortex. ×128.



Stages of penetration and progress of the cotton wilt fungus in Gadag I.

Fig. 17. Two days after inoculation. The germination of the microspore (M. C.) and the attempt of the germ tubes to penetrate through the peripheral cork (Pc) are seen. ×390.

Fig. 18. Four days after inoculation. The entry of the fungus into the epidermis after passing through the peripheral cork is seen. ×390.

Fig. 19. Six days after inoculation. The hyphae are seen in the outermost layer of the cortex. Co.—Cortex. Pc.—Peripheral cork. ×390.

Fig. 20. Seven days after inoculation. The fungus is travelling through the cortex. ×390.

Fig. 21. Eight days after inoculation. The fungus has reached the last layer of the cortex. en.—Endodermis. ×390.

The fungus was able to penetrate through the cork layer and the suberized walls of the epidermis on the fourth day after inoculation (Figs. 13 & 18). Single, solitary hypha could penetrate the layer of the peripheral cork and the suberized cell walls. Even at those places where a number of hyphae got accumulated over the outer surface of the root, only a single hypha was seen penetrating into the epidermal cells (Fig. 13). The hyphae did not show signs of swelling or shrivel-

ling on meeting with the cork layer. The penetration through the cork layer did not seem to be in any way different from that through the cell walls, though it cannot be definitely said whether the penetration was mechanical or by the dissolution of suberin into pectin-like substances. The contents of the penetrated epidermal cells were apparently undisturbed.

The progress of the fungus in both the hosts on the next day after its entry (*i.e.* fifth day after inoculation) was not very marked. It was either making its way through the epidermis or entering the first layer of the cortex (Fig. 12). The contents of the cortical cells showed no disturbance.

By the sixth day after inoculation the hyphae had crossed the first layer of the cortex and were making their way through the second layer. In only one case in Dharwar II they were just entering the third layer of the cortex. The hyphae that were travelling through the tissues were quite normal. No injury seems to have been done to them, though their progress in the two hosts was slower than that in the susceptible host upto this stage.

The progress of the fungus differed slightly in the resistant and the immune varieties on the seventh day after inoculation. In the former the rapidity of the fungus penetration was considerable. It reached the last but one layer of the cortex, while in the latter the hyphae had only crossed the second or third layer of the cortex.

The fungus was all along intracellular and not as Fahmy observed, intercellular. It reached the last layer of the cortex in the resistant as well as the immune varieties on the eighth day after inoculation. In the immnue type the fungus had barely crossed the second or third layer of the cortex till the previous day, while the next four or five layers were crossed on the eighth day. The delay observed on the seventh day was made up and the progress was nearly the same in both the varieties on the eighth day. In the resistant Dharwar II the endodermis was also penetrated in a few cases. The rapidity with which the fungus had progressed after the seventh day after inoculation in Dharwar II and on the eighth day in Gadag I points to the probability that resistance offered by the two hosts was confined more towards the outer layers, of the cortex and the epidermis. Having crossed the outer layers, it almost sprang to the endodermis as if relieved from pressure.

In fresh sections it was observed that in addition to the peripheral cork layer formed over the epidermal cells, the cells of the first and even of the second layers of the cortex, here and there were suberized by the fourth or fifth day after inoculation. The suberized cell walls might have been one of the factors of resistance observed in the outer layers of both the types, though the delay of one day to reach the endodermis in the case of Gadag I might not be of any significance.

Observations made with plants ($1\frac{1}{2}$ week old) grown in wilt-sick soil and kept in the plant-house without controlling the soil temperature, showed that the fungus made its way into the xylem vessels in the resistant Dharwar II and Gadag I accompanied by the typical discoloration of the vessels. No other changes were observed in the stele.

Resistance in relation to soil temperature.

Soil temperature is one of the most important "conditioning factors" of environment. Observations made with the temperature tanks, where a constant temperature was maintained, showed that the susceptible variety Dharwar I was very highly resistant after 32°C. Under low temperature conditions (18°—25°C.) Dharwar I was cent per cent susceptible. Dharwar II had shown a high degree of susceptibility while in the case of Gadag I there was very little wilting. The high resistance of Dharwar I at high temperatures and the breaking down of the same of Dharwar II and even of Gadag I at low temperatures made it clear that there exists a close relation between the soil temperature and the disease (wilt).

The anatomical peculiarities observed in the three types both at high and low temperature conditions throw some light on the (histological) nature of the resistance offered by the plants.

Dharwar I plants grown in infected soil and kept in cold tanks (18—25°C.) had their tap roots infected first and later on the side roots. The affected roots were never observed to rot. The epidermal cell walls, those of the cortical cells of one or two layers and sometimes even of the endodermis were suberized. It was not uncommon to find a little peripheral cork over the outer epidermal walls. When the fungus infected the side roots, there was no suberization of the outer cell walls of epidermis. In the artificially inoculated radicles and even in the infected plants from the soil the development of either the peripheral cork or suberization of the cortical cell, was confined to the portion that was attacked by the fungus. The above developments took place uniformly all round the root, only when the fungus had surrounded its whole surface.

The epidermal, cortical, endodermal and phloem cells showed no signs of disturbance in their cell contents. The typical brown discoloration of the xylem vessels was seen in all the affected plants. The amount of the fungus, judging from the number of vessels clogged partially or completely, was great.

In a plant which was six weeks old and which had resisted the disease till then, the upper (older) portion of the tap root showed secondary thickening which commenced a week before. Hyphae were seen sticking to the outer cork-layer trying to enter, but none of them gained entrance. Many of the xylem vessels were free from the fungus though the discoloration was observed in some,

PLATE XXIV.

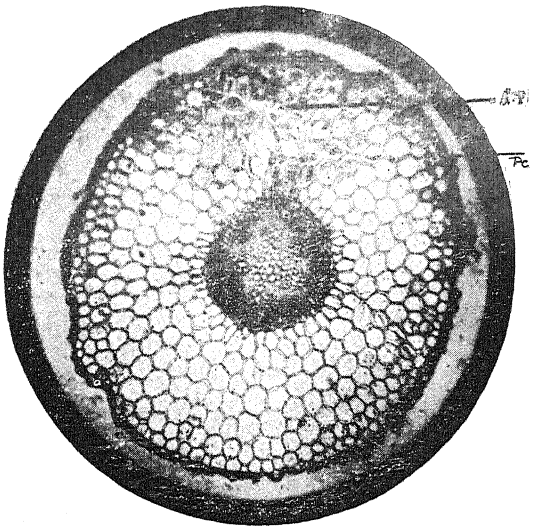


Fig. 1.

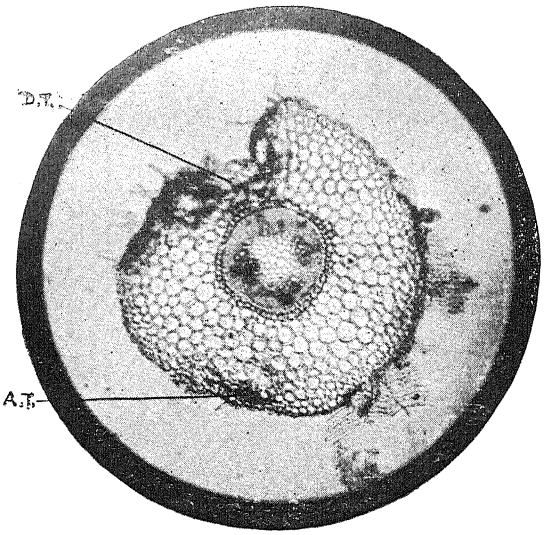


Fig. 2.

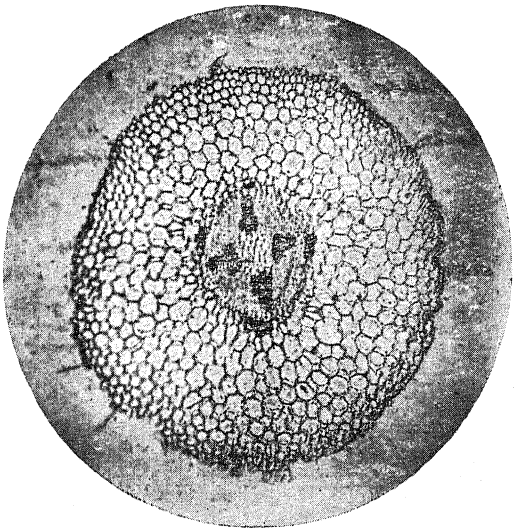


Fig. 3.

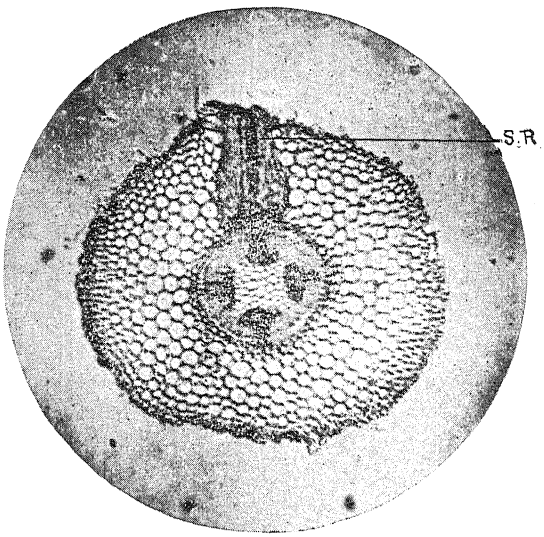


Fig. 4.

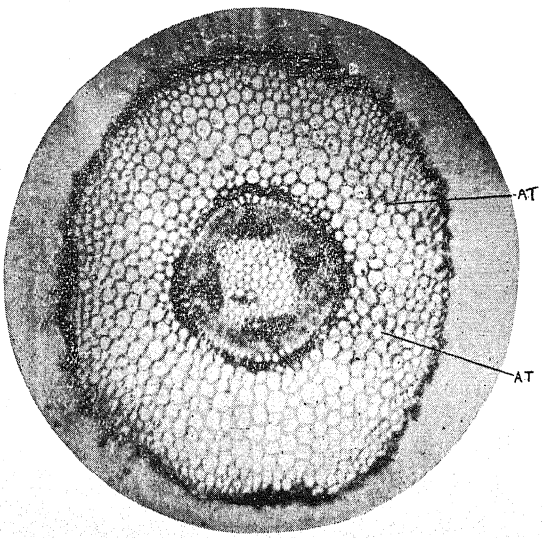


Fig. 5.

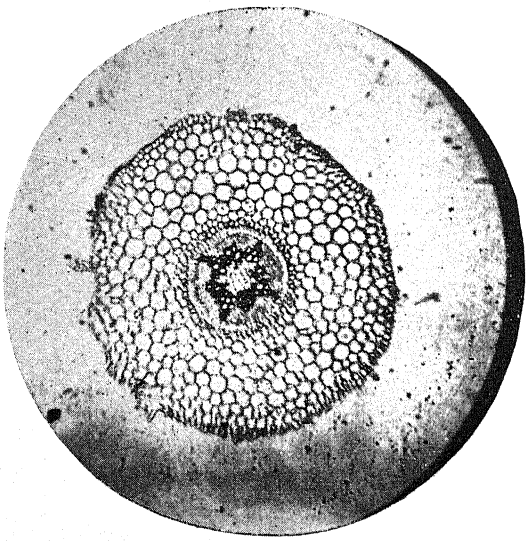


Fig. 6.

Lower, tender portions of the same root showed no secondary thickening. The fungus could make its way into them in the usual manner.

The resistant and the immune types grown under similar conditions showed the same peculiarities as had been observed in the case of the susceptible variety. The suberization of the epidermal and the cortical cells (first and second layers), the development of a little peripheral cork over the outer walls of the epidermis, the progress of the fungus without causing any injury either to itself or to the host, the presence of the fungus in the vessels accompanied by their discoloration and the early initiation of the secondary thickening were observed in Dharwar II and Gadag I. The only difference between the two varieties was that in Dharwar II the fungus was abundant where as in Gadag I it was much less.

The controls for all the three varieties Dharwar I, Dharwar II, Gadag I grown in sterilized soil and kept as the infected ones, in the cold tanks (18-25°C.) showed slight suberization of the epidermal cell walls only. The upper, older part of only a few six week old control plants showed just the beginnings of the secondary thickening.

A comparison of the infected with their controls showed that the early initiation of the secondary thickening and the pronounced suberization of the epidermal and cortical cell walls as well as the slight development of the peripheral cork were induced in the former because of the presence of the fungus. The low temperature was responsible for the breaking down of the resistance of Dharwar II and Gadag I. But the resistance of Gadag I was very great even under such unfavourable conditions.

At higher temperatures 32°-34°C. the susceptibility of Dharwar I was reduced to a minimum. The tap root was affected first and then the side roots as in the case of plants in the cold tanks. A thick layer of peripheral cork all round was observed. It was thicker where the fungus had attempted to enter the host tissues. The walls of the epidermal cells were well suberized and at times those of the cortex also. The walls of those cortical cells through which the fungus penetrated and of those in their neighbourhood were suberized and the contents of those cells became brownish yellow in colour and gave test for suberin. In a transverse section, the affected portions of the tissues appeared as brown patches (Plate XXIV, fig. 5). The affected cells were turgid and did not disintegrate after the development of suberin. The important feature was that the suberizationof the cell walls and the disturbance in the cell contents took place even before the entry of the fungus.

Only those endodermal cells that were opposite to the xylem bundles that first developed suberin in their cell walls and cell contents. Other endodermal cells were suberized a little later (Plate XXIV, fig. 5). The fungus after having made its entry into the epidermis travelled through the tissues until it reached the xylem, which it

clogged as usual accompanied by the production of the brown discoloration. Though a few plants wilted, a great many of them were successful in resisting the disease. In the apparently healthy plants the fungus did enter the xylem vessels, but it appeared to be not very abundant. All the peculiarities described above were seen in those plants also and in no way could they be said to be more pronounced. It was evident that the plants survived not by avoiding the fungus or by merely putting barriers in its way but by some inherent capacity of the protoplasm to resist the fungus attack.

The resistant and the immune varieties were better protected than the susceptible one, at higher temperatures (32-34°C.). The peripheral cork which was as thick as in Dharwar I was produced in the same manner over the attacked portion of the root at first and then all round. The cell walls of the epidermal cells as well as the cortical cells beneath the place of attack were suberized. The contents of those cells had undergone complete disorganisation which was evident from the plasmolysed dark brown matter observed in all those cells (Plate XXIV, figs. 1 & 2). The brown matter gave test for suberin. The fungus was seen entering through the cork layer outside as well as through the outer epidermal wall of the root. The penetration was extremely slow. As the fungus progressed the cell walls in front of it became suberized and the contents of those cells disorganised. The host was putting fresh barriers in the way of the fungus, as it succeeded in pushing its way inside. Progress of the fungus through the tissues depended on its quickness in travelling through the disorganised cells before they collapsed. It rarely crossed the endodermis which had also developed suberin in its cell walls and cell contents. But no plasmolysis of its contents had taken place. Where the fungus succeeded in getting into the inner stele, the phloem and parenchymatous cells were also suberized. The vessels turned dark brown. In those cases the brown walls of the vessels gave test for suberin. The cells, both epidermal and cortical, which were affected collapsed after a time and disintegrated. The hyphae that could enter the stele were very few, and clogging of the vessels was uncommon in both the hosts. The portion which was free from the attack of the fungus showed neither suberization of its cell walls nor any disturbance in its contents.

It is evident that in both the resistant and immune varieties, the fungus was very effectively checked from making much progress in most of the cases, by the disorganisation of the cell contents first, and the disintegration of that part later on. Fungus could do no harm even where it gained entrance into the stele. Resistance of Dharwar II and Gadag I consists in their ability to develop suberin in their cell walls and the ready response observed in the cell contents of the affected cells.

The controls for the three varieties in these high temperature experiments showed no such disturbances in their tissues, but the epidermal walls of all the

types were slightly suberized as in the controls in the cold tanks (Plate XXIV, figs. 3, 4 & 6).

In the absence of the high temperature and in the presence of the fungus, the three varieties had shown greater susceptibility. There was neither a thick peripheral cork layer nor the disturbance in the cell contents of the tissues. The absence of the fungus and the increase of temperature had not resulted in producing the changes observed in the infected plants at high temperatures. It was evident from these facts that the high temperature was responsible for the great resistance offered by the susceptible variety. Dharwar I was extremely resistant while Dharwar II and Gadag I were immune under these conditions.

Discussion.

The present study was undertaken to see how the pathogene penetrates and progresses in the susceptible, the resistant, and the immune varieties of cotton; to see whether the above mentioned three types of cotton could be distinguished by their structural characters and lastly to see how they behave at high and low temperature conditions towards the causal organism, *Fusarium vasinfectum*.

The behaviour of the fungus towards the three types, that differed markedly in their capacity to resist was to a great extent similar under the plant-house conditions.

The germination of the spores in all types was delayed till the second day after inoculation. The germ tubes entered the root directly or they first elongated and spread over its surface. In the case of the cotton seedlings, the entrance into the root was effected by the germ-tubes or the hyphae, so far as observations show, by direct penetration of the epidermal cells only.

The question of fungus entry through the cell wall has been under study for a long time. Marshall Ward [1902] who was one of the earliest to study it, suggested that it might be due to the dissolution of the cell wall at the point of infection by the parasite in contact with it.

Blackman and Welsford [1916] had shown that *Botrytis cinerea* did not secrete a cutin-dissolving enzyme but penetrated the cuticle by mechanical means alone.

Brown and Harvey [1927] found that "the germ-tubes of *Botrytis* were unable to penetrate the epidermis of *Eucharis sp.* and certain other plants until the turgor of the underlying cells was reduced by plasmolysis". They also found that in "the penetration of the membranes by the fungi, the stimulus was one of contact and the means of penetration was purely mechanical".

It is difficult to say anything definitely in the case of cotton. The absence of any abnormality in the contents of the penetrated cells indicates that penetration was effected not as a result of any injury to or change of the protoplasmic contents

of those cells. There was no plasmolysis of the cell contents to suggest that entrance was gained because of the removal of turgor. The penetrating hyphae were normal before and after the entry into the host. The cell wall surrounding the place of entry did not appear to be thin or weakened. There was not more than one hypha entering through the same point.

In the case of the resistant and the immune types, the pathogene could gain entrance into the epidermal tissues on the fourth day as in the case of the susceptible type. The penetration, in spite of the presence of the peripheral cork layer, was similar to that in the susceptible host.

Conant [1927] in his study of the resistance of tobacco to *Thielavia basicola*, had not observed the penetration of the suberized walls in the tobacco roots by a single unaided hypha. The penetration of such cell walls occurred as a result of a sort of "mass action", where a weft of hyphae came to lie in contact with the walls, which were probably weakened by the enzymes secreted. "A number of hyphae then appear to surge through the weakened walls."

In the case of cotton, single hyphae could penetrate through the layer of cork and suberized cell walls. Even where a number of hyphae came to lie on the outer surface of the root, the penetration was effected only by a single hypha. From the observations, it seems clear that penetration of the fungus into the cotton seedlings is mechanical, *i.e.*, by the piercing of the cell wall.

In the susceptible variety the further progress of the fungus was one of invasion of layer after layer of cells, until it reached the xylem, which it entered after the eighth day of inoculation.

The resistant and the immune types differed from the susceptible host in that the progress of the fungus was retarded to a certain extent in the outer layers of the root, *i.e.*, the epidermis and one or two layers of the cortex. By the fourth day after inoculation a layer of peripheral cork, which varied in its thickness in accordance with the severity of fungus attack, was formed over the outer walls of the epidermis. The walls of the epidermis and even of the cortical cells here and there of the outer one or two layers were all suberized. The fungus could go only to the first or second layer of the cortex in Dharwar II and Gadag I by the sixth day after inoculation, while it had gone half way into the cortex by that time in Dharwar I.

Though the delay in progress was considerably made up during the following two days in Dharwar II and Gadag I, yet it could reach the endodermis only by the eighth day, by which time it had already entered the xylem vessels in Dharwar I. The fungus was meeting with resistance, while trying to make its way into the tissues in Dharwar II and Gadag I, was certain. Whether the resistance was because of the suberization of the cell-walls or the difference in the physiological nature of the cell-contents is not definitely known.

Tisdale [1917] working on flax wilt (*Fusarium lini*) reported that the thickened walls would not be sufficient by themselves to prevent invasion in flax plants resistant to wilt. He believed that some toxic or other chemical substance was produced by the protoplasm of the resistant host, which had a deleterious effect on the fungus and concluded that resistance of flax to *Fusarium lini* was essentially of a chemical nature.

Gilchrist [1926] found that resistance to foot rot in pea was due to the cuticle being thicker in the resistant variety than in the susceptible one.

In the case of the tobacco root rot, the fungus *Theilavia basicola* was found to grow and sporulate as freely in the resistant as in the susceptible one. Conant [1927] observed that resistance to root rot in tobacco "was definitely correlated with the ability of the host to develop a cork layer beneath the point of infection". He concluded that unaided by the cork formation, the special chemical substance or character, that the resistant tobacco roots might be containing, would be of no avail.

Studying the parasitism of *Colletotrichum Lindemuthianum*, Leach [1923] observed that resistance was due to the inability of the fungus to obtain nourishment from the living protoplast.

Weimer [1929] found that the layer of cork formed over the wounds in sweet potatoes was "not alone responsible for the protection of the wounds ; though the suberization of the surface cell-walls was a factor preventing the entrance of the micro-organisms ".

In cotton, under ordinary conditions of atmosphere, the fungus was able to penetrate and progress in the roots of the susceptible, the resistant and the immune types, though in the latter two some slight resistance was offered. The development of a peripheral cork layer or of suberin in the cell walls of the epidermal or cortical cells could not check the fungus entry or its further progress, though it is possible that they might have been some of the factors that the fungus had to overcome in order to gain entrance. It appears that resistance in cotton might be due to the combined action of both the production of cork over the surface and the cell walls, and the resisting quality of the protoplast.

The response of the three hosts to the varying conditions of temperature was very interesting. The resistance of Dharwar II was broken to a very great extent under low temperature conditions (18- 25°C.). But the immune variety was very highly resistant even under those conditions, which shows that the resisting quality was so great that it could not be broken to any large measure.

The suberization of the epidermal cell walls was common to all the types irrespective of the fluctuations in temperature, the degree of resistance and also the presence or absence of the fungus.

The infected plants of Dharwar I, Dharwar II, and Gadag I grown in wilt-sick soil and kept in cold tanks showed suberization of the epidermal walls and in addition a little peripheral cork at points of severe attack and also the suberization of the cortical cell walls of one or two outermost layers. The peripheral cork, however little, and the suberization of the cortical walls seem to be produced in response to the attack of the fungus entirely, though these developments were found insufficient to successfully prevent the fungus from entering the host tissues. The rapid progress of the fungus and the healthy appearance of the tissues, other than the xylem, in Dharwar I and Dharwar II under low temperature conditions, point to the fact that practically little or no resistance was offered by the cell contents of those cells. Gadag I which resisted better than Dharwar I and Dharwar II developed no thicker layer of peripheral cork nor was there any other histological peculiarity that could account for the resistance observed in it.

Dharwar I notwithstanding the thick layer of cork produced outside the epidermis, the suberization of the cell walls of the epidermis, cortex and endodermis, and the disorganisation accompanied by the development of suberin by the cell contents at high temperatures could not effectively check the invasion of the fungus. While in Dharwar II and Gadag I the fungus could not make any headway on account of the complete disorganisation of the cells surrounding the place of infection and complete collapse and disintegration of that part later on.

If the peripheral cork and the suberized cell walls were really effective in preventing the fungus from making its way through them, no fungus could be expected even in the susceptible host under low temperatures also. But the results show that it did enter all the hosts whatever the degree of resistance might be, under all conditions and in spite of barriers put in its way in the shape of suberized cell walls inside and peripheral cork outside.

The anatomical features alone cannot explain the varied degree of resistance seen in Gadag I at low temperatures and of Dharwar I at high temperatures. Dharwar II and Gadag I which were immune at high temperatures had the thick peripheral cork, suberized cell walls, and the disorganised cell contents. If the absence of these features to the same extent in low temperatures can account for their susceptibility under those conditions, then Gadag I should have succumbed to the disease to the same extent as the susceptible Dharwar I which was as badly protected.

Again if these features were alone responsible for the high resistance of Dharwar II and Gadag I at high temperatures, then there was no reason why even a small percentage of Dharwar I plants, which were equally well protected by the development of all these anatomical features, should succumb. There can be no doubt that the development of the peripheral cork and the suberization of the epidermal and

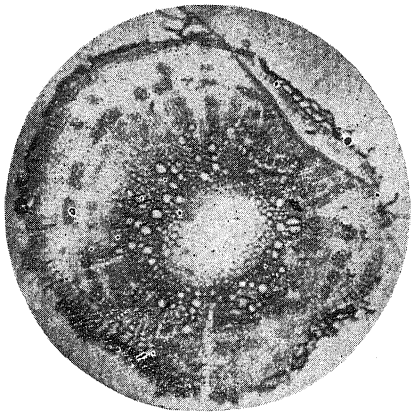


Fig. 1.

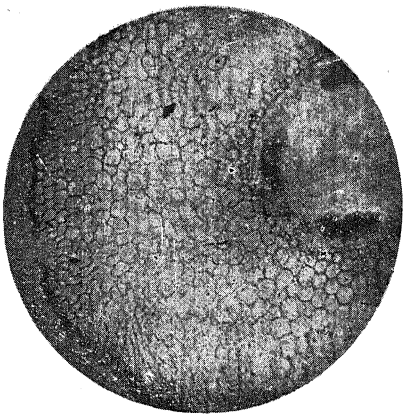


Fig. 2.

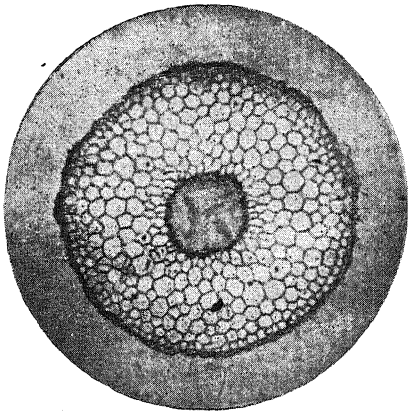


Fig. 3.

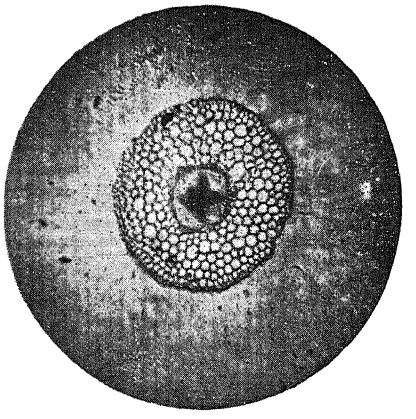


Fig. 4.

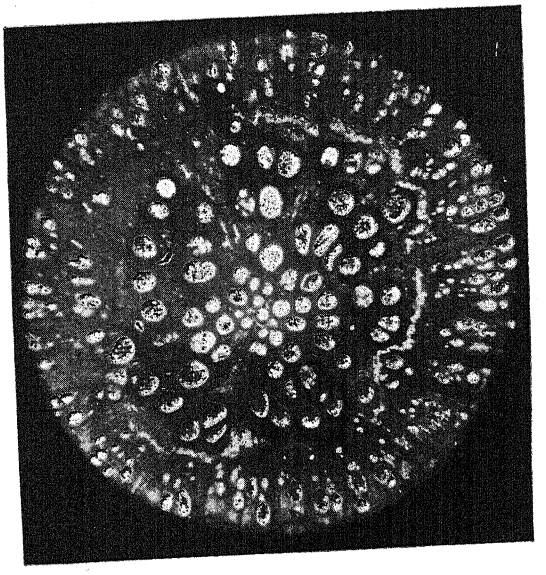


Fig. 5.

(*For explanation please see p. 313*).

the cortical cell walls can put up great resistance, though it may be only temporary. In the older parts of the tap roots of all the varieties where the cork cambium and the thick peripheral cork were formed, the fungus was not observed to make its way into the tissues. But by the time the secondary thickening took place the fungus had already reached the xylem vessels and hence it could not be of great advantage. It is clear that the peripheral cork and the suberized cell walls do not constitute the main cause of resistance in cotton.

Mention had been made in the preceding pages that the disturbance in the cell contents of the affected tissues of the roots of all the varieties at higher temperatures was the result of the increase in temperature. The absence of the above reaction seems to have something to do with the increased susceptibility of Dhawar I, Dharwar II and the breaking of the immunity of Gadag I at low temperatures. It seems very likely that the resistance at high temperature was connected with the ability of the host protoplasm to respond to the stimulus of the parasite. Whether the reaction was due to the sensitiveness or any other property of the protoplasm cannot be said with any certainity.

Summary.

1. The mode of entry of the parasite and the manner of its progress are the same in all the varieties of cotton—susceptible, resistant, immune—irrespective of their capacity to resist.

2. Single hypha was seen to make its way successfully through the peripheral cork and suberized cell walls in Dharwar II and Gadag I, without a weft being formed.

3. In Dharwar I, the susceptible type, the fungus made its way into the epidermis on the fourth day and reached the xylem on the eighth day after inoculation.

4. In the resistant Dharwar II and immune Gadag I, though the entry was on the fourth day as in Dharwar I, it could just enter the endodermis or reach its outer walls by the eighth day.

5. At the ordinary temperatures of the plant-house the resistant and the immune types were seen to develop peripheral cork first over the infected portion and later over the other parts of the root, as the fungus encircled it.

6. At low temperatures like 25°C. or below, the susceptibility of Dharwar I and Dharwar II greatly increased. Even the immune type had shown susceptibility to a slight extent.

7. The infected plants of these types at low temperatures showed the suberization of the epidermal and cortical cell walls (one or two layers). A little peripheral cork was also developed more prominently under the points of infection.

8. The initiation of secondary thickening of the root in the infected plants was much earlier than in the controls.

9. The controls showed only the suberization of their epidermal walls.

10. At higher temperatures like 32-34°C. the susceptible type resisted to a greater extent and Dharwar II and Gadag I showed no deaths.

11. The peripheral cork was thick in the infected roots of all varieties at high temperatures. The epidermal cells all round and cortical cells directly below the place of attack as well as those in their neighbourhood were not only stimulated to develop suberin in their walls in advance of the approach of the fungus, but their cell contents were also disorganised to a greater or less extent in all the varieties.

12. The controls of these showed nothing but slight suberization of the epidermal cells.

13. The development of peripheral cork and the suberization of the cells do not constitute the main cause of resistance, though they could not be dispensed with.

14. Resistance in cotton seems to be due to the nature of the host protoplasm mostly.

ACKNOWLEDGMENT.

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The writer is indebted to Mr. G. S. Kulkarni, and the staff of the Cotton Research Laboratory, Dharwar, for assistance of various kinds during the progress of this investigation.

REFERENCES.

Ajrekar, S. L. (1926). *J. Ind. Bot. Soc.* **5**, 1-8.

Ajrekar, S. L. and Bal, D. V. (1921). *Agric. J. Ind.* **16**, 598-617.

Allen, R. F. (1923, 1). *J. Agric. Res.* **23**, 131-152.

————————(1923, 2). *J. Agric. Res.* **26**, 571-604.

Blackman, V. H. and Welsford, E. J. (1916). *Ann. Bot.* **30**, 389-398.

Brown, W. and Harvey, C. C. (1927). *Ann. Bot.* **41**, 643-662.

Butler, E. J. (1910). *Dept. Agric. Ind., Mem. Bot. Series* **2**, 9.

Butler, E. J. (1926). *Agric. J. Ind.* **21**, 268-278.

Butler, E. J. (1918). Fungi and diseases in plants. (Thacker Spink & Co., Calcutta.)

Chamberlain, C. J. (1924). Methods in plant histology. (Chicago Uni. Press.)

Conant, G. H. (1927). *Amer. J. Bot.* **14**, 457-480.

Dastur, J. F. (1924). *Agric. J. Ind.* **19**, 251-260.

Fahmy, T. (1928). Minis. Agric. Egypt. Tech. Sci. Series Bul. **74**.

————————(1930). Etude de la penetration du champignon *Fusarium vasinfectum* (Atk.) *var ægypticum*. T. Fahmy dans les racines du cotonnier. Uni. de Geneve. Inst. de. Bot. These. **881**.

Gilchrist, G. (1926). *Phytopath.* **16**, 269-276.
Gilman, J. C. (1916). *Ann. Missouri Bot. Gard.* **3**, 75-84.
Jones L. R., Johnson, J. and Dickson, J. G. (1926). Agric. Expt. Sta. of Uni. Wis. & U. S. Dept. Agric. Res. Bul. 71.
Kulkarni, G. S., and Mundkur, B. B. (1928). *Dept. Agric. Ind., Mem. Bot. Series.* **17**, 2, 128.
Leach, J. G. (1923). Min. Agric. Expt. Sta. Tech. Bul. 14.
Neal, D. C. (1927). *Ann. Missouri Bot. Gard.* 14, 359-424.
Orton, W. A. (1900). U. S. Dept. Agric. Div. Veg. Physiol. and Path. Bul. 27, 1-16.
————(1908). U. S. Dept. Agric. Year book, 453-464.
Peltier, G. L. and Rayburn, W. S. (1926). U. S Dept. Agric. Bul. 1417.
Shaw, M. F. (1929). *Amer. J. Bot.* **16**, 259-272.
Smith, R. and Walker, J. C. (1930). *J. Agric. Res.* **41**, 17-35.
Tisdale, W. H. (1917). *J. Agric. Res.* **11**, 573-607.
Vavilov, N. T. (1919). Immunity of plants to infectious diseases, Proc. of the Petrovsky, Agr. Acad. 1918, 1-239. (with a resume in English).
Walker, J. C. (1924). On the nature of disease resistance in plants. Trans. Wis. Acad. Sci. Arts. and Letters. 21, 225-247.
Weimer, J. L. and Harter, L. L. (1921). *J. Agric. Res.* **21**, 637-647.
Ward, H. M. (1902). *Ann. Bot.* **16**, 233-315.

Explanation of plates.

Plate XXIV.

1. Section of the infected root of Gadag I from the incubator (32°C.-34°C.) Note the varying thickness of the peripheral cork and the difference between the affected and healthy portions of the tissues.

The plasmolysed contents of the affected cells can be seen clearly. This is the stage before the complete disintegration of that tissue later on.

A. T.—Affected tissue.; P. C.—Peripheral cork.

2. Section of the infected root of Dharwar II from the incubator. Note the disintegrated tissue. D. T.—Disintegrated tissue.
3. Control for Gadag I.
4. Control for Dharwar II. S. R.—Side root.
5. Section of the infected root of Dharwar I from the incubator. Note the affected tissue. The contents of the affected cells are turgid as the healthy ones.
6. Control for Dharwar I.

Plate XXV.

1. Section of the infected root (six weeks old) of Dharwar I. Note the secondary thickening.
2. Section of the root of a control plant (Dharwar I) which was of the same age as the infected one. There is no secondary thickening.
3. Section of the infected root of Dharwar I from the cold tank (18°—25°C.).
4. Control for Dharwar I from the cold tank.
5. Section showing the typical discoloration of vessels and their clogging by the hyphae. Discoloured vessels are very dark in the photograph.

SELECTED ARTICLES.

SOME NEGLECTED SOIL FACTORS IN PLANT GROWTH[1].

BY

A. H. MEYER[2].

(With Plates XXVI & XXVII.)

(Reprinted from the *Journal of the American Society of Agronomy*, Vol. 23 No. 8, August 1931.)

The difficulty of growing normal plants in synthetic media is one of common experience. The reason is self-evident, for all the factors that influence plant growth are not well understood. In the matter of nutrition alone there is increasing evidence that more than 10 of the 80 odd chemical elements known are essential and indispensable to the life and growth of green plants. Some elements have apparently been overlooked owing to the fact that they are required in such small amounts, that either they have been supplied accidentally as impurities in the nutrient salts, or else the growth of the plant was continued for such a short period that the needs were supplied by small amounts stored in the seed.

Aside from the additional elements which may be essential for plant growth, there are factors connected with possible toxic conditions which may be important. Certain kinds of finely divided or colloidal material, such as kaolin, silica, and ferric oxide, which are found in soils may, when added to culture media, combine chemically or physically with toxic material produced in the culture media or excreted by plant roots, thus inhibiting the action of the toxins and greately favoring root development and plant growth. That there may be factors of this kind is indicated by observations to the effect that plants usually grow better in a good soil than in the best artificial medium that may be prepared.

It was the purpose of this investigation to study some of the more or less neglected soil factors in plant growth which have just been mentioned.

Review of literature.

Of the additional elements sometimes said to be essential, manganese has been given the most consideration. It is invariably present in soils and also in a large variety of plants and animals. In the analyses of 23 Italian soils reported by

[1] Abstract of a thesis submitted at the University of Wisconsin in partial fulfilment of the requirements for the degree of doctor of philosophy. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Received for publication February 28, 1931.

[2] Associate professor of Soil Research, Louisiana Agricultural Experiment Station, Baton Rouge, La. The writer wishes to express his appreciation for the helpful suggestions and criticisms tendered by Professor E. Truog, under whose general direction this work was done.

Contino (*6*)[3] the amounts of manganese oxide range from a trace to as high as 0·48% the average being 0·17%. In Kentucky soils, according to Shedd (*19*), the percentage of manganese ranges from 0·05 to 0·331% in the surface soil and from 0·002 to 0·264% in the subsoil. He found that a high percentage of manganese is associated with rich soils, that the cultivated soils contain less manganese than the virgin soils, and that under cultivation manganese is usually lost more rapidly than phosphorus.

Jardin and Astruc (*10*) examined about 65 species of plants and found manganese in all of them. Their work also indicated a wide distribution of the element in the animal kingdom.

McHargue (*14*) found the largest amounts of manganese in those parts of the seed coats that immediately surround the cotyledons. He found, too, that manganese is relatively high in those parts which secrete large amounts of oxidizing enzymes. He indicates that manganese may serve as a catalyst to the enzymes.

Bertrand (*1*, *2*) was probably the first worker to assign a definite roll to manganese in plant nutrition. In pot experiments manganese increased the yield from 10 to 20% while in the field the increase ranged from 20% with peas to 30% with alfalfa. He concluded that manganese is apparently not to be replaced by another element, not even iron, and that just because it occurs in small amounts is no reason for regarding it as a secondary element in plant nutrition.

Brenchley (*4*, *5*), from work with pot cultures, concluded that manganese may be an essential element for plant nutrition, and noticed that it is toxic only in large amounts. Minute traces of this element in the from of a salt were found to have a very beneficial effect on both roots and shoots.

In 1916, Skinner and Reed (*20*) reported the results of a 6-year test in which 50 pounds of manganous sulfate per acre were applied on an acid and a neutral soil. On the neutral soil they received increased yields of wheat, rye, corn, timothy, beans and cowpeas, but not potatoes. There was no apparent benefit on the acid soil. They concluded that on acid soil, manganese decreased the oxidation power, while on neutral soil it increased it.

Hiltner and Korff, in 1925, concluded that manganese is only beneficial to plants in that it accelerates oxidation in the soil. Two years later McLean and Gilbert (*17*) showed that manganese is a cure for chlorotic spinach.

McHargue (*15*) argues that the statement, "the 10 essential elements are the only ones that have important functions in the growth of plants" is no longer tenable. He states that small amounts of manganese, copper, zinc, boron, barium, strontium, iodine, and arsenic are normal constituents of plants grown under natural

[3] Reference by number is to "Literature Cited", pp. 330—1.

conditions on the fertile soils of Kentucky. Manganese, though less abundant, is as widely distributed as iron. Just lately McHargue and Shedd (*16*) have published data giving further support to these ideas.

Maze (*13*) found that it is impossible to grow plants in a culture solution without the addition of small amounts of several of the elements rare in the ash of plants. Sommer and Lipman (*21*) obtained evidence that silicon, aluminium, boron, zinc, and chlorine are essential.

From a study of the literature it appears that there is a growing opinion that other elements than the so-called 10 essential elements are necessary for the normal growth of plants. It is natural that investigators should first have centered their thoughts on the elements occurring in the largest amounts in plants. The apparently lesser important elements are required in such small amounts that they were undoubtedly often furnished in sufficient quantities as impurities in the nutrient salts and thus their presence or need were overlooked. Even the necessity of iron was overlooked until Knop's investigation, a little over a half century ago. With the development of new and more accurate methods of chemical analyses and with greater refinement in methods of research, it seems entirely probable that some of the elements now called unessential may prove to be essential and very important in the metabolism of plants.

Another factor involved in plant growth is that of toxins. Nageli (*18*) discovered as early as 1893 that by adding crushed graphite, shredded filter paper, paraffin shavings, or other insoluble substances in a finely divided state to the culture medium the toxic effects of copper were entirely inhibited.

Dandeno (*7*) found that the finer the sand is in the culture medium the more pronounced is the reduction of the toxic effect of copper sulfate. Similar results were obtained by Jensen (*11*) with sulfuric acid.

In 1905, True and Oglevee (*22*) observed that the toxicity of solutions of silver nitrate and copper sulfate were materially reduced by the addition of such insoluble substances as quartz sand, powdered glass, shredded filter paper and starch grains to the culture. Breazeale (*3*) on the other hand, in testing the toxicity of sulfuric acid to maize seedlings, was unable to reduce this toxicity by the introduction of quartz flour, filter paper or paraffine to the solution. However, with copper sulfate the medium was materially ameliorated by the addition of carbon black.

Jensen (*11*) found that quartz flour reduces the toxicity in most cases, but not when due to phenol and alcohol which, like resorcinal, are very week poisons to plants. His results are fully in agreement with those of True and Oglevee (*22*).

Livingston's (*12*) data reveal that all poor soils studied by him, as well as the aqueous extracts of these soils, were appreciably improved by the addition of calcium carbonate. Similar results were obtained by Breazeale (*3*).

The data of Breazeale bring out the point that the effects of calcium carbonate and ferric hydrate (chemical agents) are much more pronounced than are those of carbon black and quartz flour (physical adsorptive agents.)

Funchess (*8*) reports that a normal soil can apparently dispose of enormous quantities of organic compounds through physical, chemical, and biochemical action.

In 1916, Truog and Sykora (*23*) made a study of soil constituents which inhibit the action of plant toxins. The insoluble materials used were quartz sand, quartz flour, kaolin, and Superior clay. Their data indicate that in the inhibition of toxicity, chemical reaction is a more important factor than physical phenomena, such as adsorption.

Experimental.

Influence of kaolin and other soil material on plant growth.

Kaolin is widely distributed and is found in considerable amounts in most soils. It seemed desirable, therefore, to study its possible influence on plant growth. In some preliminary experiments the effect of adding 1 per cent. of kaolin to quartz sand cultures of oats which otherwise received a complete nutrient solution was studied. The results secured were as follows :—

Treatment	Weight of seeds in grams	Weight of dry tops in grams	Weight of dry roots in grams
Control	5·048	10·93	4·23
100 grams kaolin	8·35	12·20	5·59

These results indicate that some factors affecting plant growth, but usually neglected are connected with the addition of koalin. In order to determine what these factors might be, more extensive experiments were planned the following year. As regards the factors involved there appeared to be at least three possibilities, *viz.*, (*a*) that kaolin contains as impurities small amounts of additional elements necessary for plant growth ; (*b*) that kaolin in some way benefits plant growth, possibly by combining with toxic material of a basic nature ; and (*c*) that the colloidal property affects plant growth through physical means.

A very high grade of natural quartz sand, analyzing over 99 per cent. silica, was used for the synthetic sand cultures. The koalin used was fairly pure material from Dry Branch, Georgia. Only the portion passing a 100-mesh sieve was used. In order to study the influence of other non-nutrient soil material than kaolin on plant growth, some Miami silt loam which had been cropped until low in fertility

was added to some of the cultures. It had a reaction of pH 6·5. The soil was passed through a 100-mesh sieve before being mixed with the quartz sand.

High tested chemicals were used to make a basal nutrient solution according to the following formula :—

Salts used	Grams per liter of solution
$NaNO_3$	0·625
$Na_2HPO_4.12H_2O$	0·462
KCl	0·382
$Ca(NO_3)_2.4H_2O$	0·240
$MgSO_4.7H_2O$	0·075
$FeCl_3.6H_2O$	0·014

Earthenware glazed pots of 2-gallon capacity were filled with 11 kilograms of quartz sand. One liter of the nutrient solution was added to each jar. The moisture content was brought up to 13 per cent. and maintained there until the crop was harvested. The reaction of the nutrient solution was pH 7·1.

Alfalfa, buckwheat, oats, and corn were grown with five different treatments as follows :—

1. Control.
2. 100 grams kaolin.
3. 200 grams kaolin.
4. 400 grams kaolin.
5. 400 grams of Miami silt loam.

The control quartz culture received only the basal nutrient solution. In the case of oats, an additional pot was added containing 10 kilograms of quartz sand and 1 kilogram of kaolin. The kaolin and sand were mixed thoroughly in the dry condition.

After the plants were up, they were thinned to 10 of buckwheat, 9 of oats, 12 of alfalfa, and 3 of corn. The alfalfa was inoculated. The pot cultures were placed in the greenhouse and kept at an approximately constant moisture content by the addition of distilled water from time to time.

The crops were all sown February 23 and excepting oats were harvested April 30. Oats were harvested May 13.

Alfalfa was the first crop to show differences in growth. After the elapse of 10 days the plants in the sand cultures with kaolin were decidedly better, having larger leaves which were also darker green in color. The plants grown in quartz sand alone were spindling and yellow in color. The addition of 400 grams of Miami silt loam did not act as favorably as 100 grams of koalin. Phosphorus starvation

was suspected and an application of phosphorus to one of the duplicates produced a very quick response, thus substantiating the surmise that phosphorus was the limiting factor. Apparently the soluble phosphate was tied up to some extent by the soil.

The alfalfa roots in the quartz sand culture had very few nodules, whereas those in the quartz sand with kaolion and in the quartz sand with Miami silt loam had a large number of nodules, and, in addition, the root development was much better.

The weights of air-dry tissue produced, given in Table I, show the beneficial effect of kaolin and Miami silt loam on plant growth in quartz sand cultures.

TABLE I.

Effects on the growth of alfalfa of the addition of kaolin and Miami silt loam to quartz sand cultures.

Pot No.	Treatment	Weight of dry tops in grams, ave. of two	Weight of dry roots in grams, ave. of two
1	Control	2·00	1·65
2	100 grams kaolin	4·50	5·05
3	200 grams kaolin	4·60	5·03
4	400 grams kaolin	5·01	4·50
5	400 grams Miami silt loam	4·70	3·85

It is evident that the kaolin had a very beneficial effect on root development. This effect is not nearly as evident with Miami silt loam.

There were noticeable differences in the growth of buckwheat with the various treatments which showed up about two weeks after planting. The plants in the quartz sand culture were spindling and the leaves pale green in color, while the plants in the cultures with quartz sand and kaolin and quartz sand and Miami silt loam were thicker in the stalk and darker green in color. At all times the best growth appeared in the quartz sand culture with 400 grams of Miami silt loam. This was contrary to the observations with alfalfa. This might be explained by the fact that buckwheat can utilize difficultly soluble phosphates more readily than alfalfa. Just before inflorescence, the plants in the quartz sand culture were drooping, the stalks spindling, and the leaves small and pale green. On the other hand, those grown in the quartz sand culture with kaolin were rigid, the stalks thick, and the leaves larger and darker green.

The comparative growth of buckwheat with the various treatments is shown in Plate XXVI, fig. 1. The weights of seeds, tops, and roots are given in Table II.

TABLE II.

Effect on the growth of buckwheat of the addition of kaolin and Miami silt loam to quartz sand cultures.

Pot No.	Treatment	Weight of seeds in grams, ave. of two	Weight of dry tops in grams, ave. of two	Weight of dry roots in grams, ave. of two
1	Control	1·10	3·75	0·24
2	100 grams kaolin	2·51	5·15	0·70
3	200 grams kaolin	3·53	4·20	0·75
4	400 grams kaolin	3·23	5·95	0·70
5	500 grams Miami silt loam	4·24	4·10	0·45

The data in Table II are in agreement with the observations made of the general appearance of the plants, with the exception of the plants grown with Miami silt loam. In this case, the weight of the tops, contrary to expectation, was not the heaviest. As in the case of alfalfa, the root development was the largest in the quartz sand with kaolin. There was no significant difference in plant growth with varying amounts of kaolin.

When it comes to the total weight of seed produced, the general appearance of the plants was a true criterion. The weight increased sharply from set 1 to set 2 and gradually from set 2 to set 5. Only in set 4 did the weight of seed exceed that of the tops.

In the series of experiments with oats the quartz sand cultures with kaolin and with Miami silt loam gave a superior growth to that of the quartz sand culture alone. At the end of two weeks' growth, the oats in the quartz sand were spindling and the leaves pale yellow, whereas, with the treatments of kaolin they were stalky and dark green in color. In the kaolin series, the oats with 400 grams of kaolin were the tallest. The plants grown in quartz sand cultures with Miami silt loam were shorter and had thinner stalks and paler leaves than those grown in quartz sand with kaolin.

At harvesting time the plants grown in quartz sand with 1,000 grams of kaolin were the tallest and had the thickest stalks and darkest colored leaves of any in spite of the somewhat retarded growth at the time the photograph was taken.

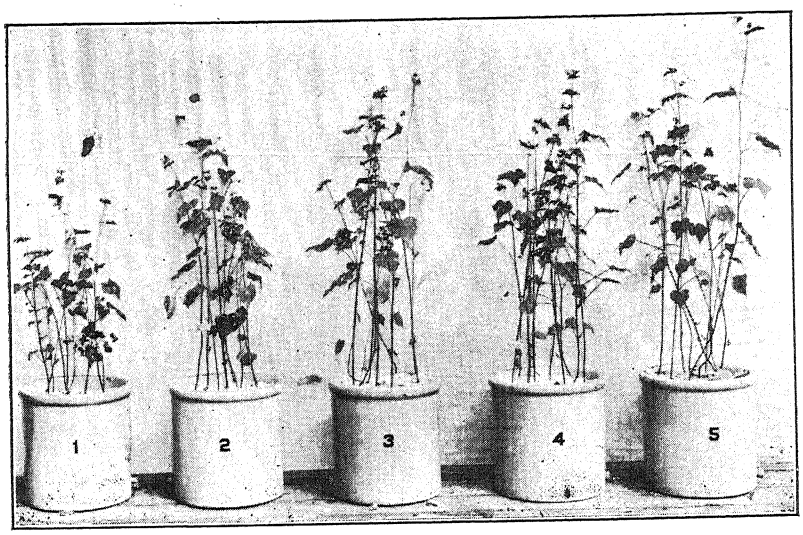


Fig. 1.—The growth of buckwheat in quartz sand culture with the addition of kaolin and Miami silt loam. 1.—Control. 2.—100 grams unwashed kaolin. 3.—200 grams unwashed kaolin. 4.—400 grams unwashed kaolin. 5.—400 grams Miami silt loam.

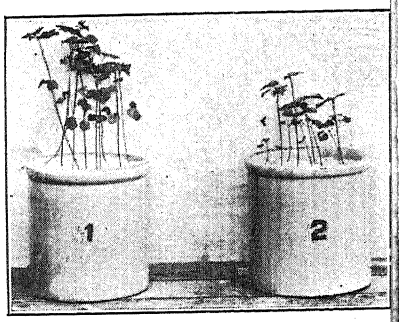


Fig. 2.—The effect on the growth of buckwheat of the addition of washed kaolin to quartz sand culture. 1.—[illegible] grams washed kaolin. 2.—Control.

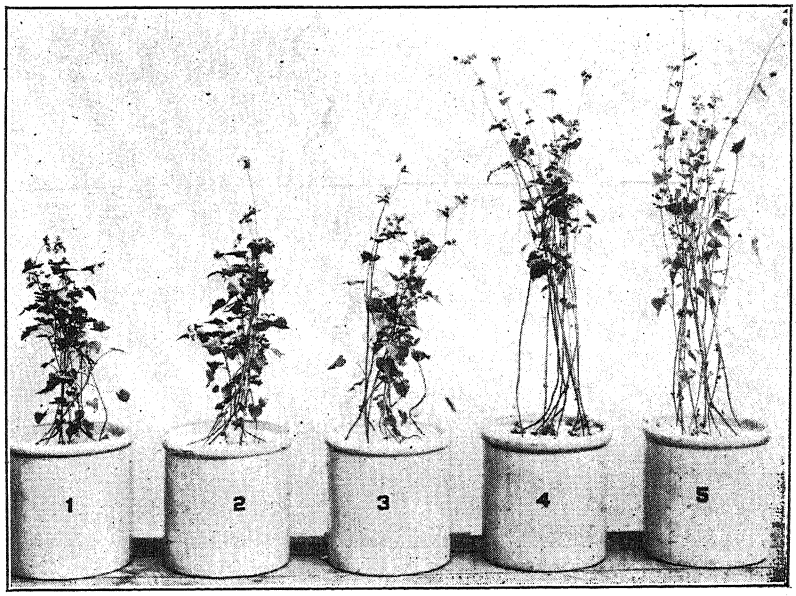
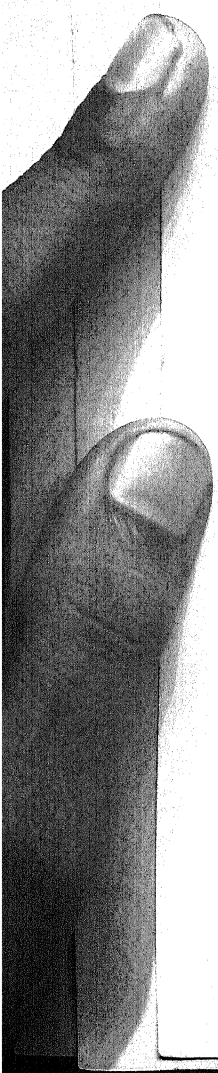


Fig. 3.—Various effects of kaolin and Miami silt loam on the growth of buckwheat. 1.—Control. 2.—Basal nutrient solution treated with 200 grams soil. 3.—Basal nutrient solution treated with 20 grams kaolin. 4.—400 grams Miami silt loam with basal nutrient solution. 5.—200 grams kaolin with basal nutrient solution.



The dark green color was especially noticeable in contrast to the plants in the quartz sand cultures. The weights of tops and roots are given in Table III.

TABLE III.

Effect on the growth of oats of the addition of kaolin and Miami silt loam to quartz sand cultures.

Pot No.	Treatment	Weight of dry tops in grams, ave. of two	Weight of dry roots in grams, ave. of two
1	Control	6·00	1·51
2	100 grams kaolin	10·90	4·95
3	200 grams kaolin	13·10	6·25
4	400 grams kaolin	11·60	5·95
5	400 grams Miami silt loam	11·00	4·50
6	1,000 grams kaolin	17·10	6·40

As indicated in Table III, the kaolin had a very beneficial effect on the root development of the oats, as it did in the case of alfalfa and buck-wheat. The heaviest root growth was obtained with 200 or more grams. The highest weight of tops was secured where 1,000 grams were used, though very beneficial results were obtained from lesser amounts. The plants grown in quartz sand cultures with 400 grams of Miami silt loam were not nearly as good as those grown in quartz sand with the different amounts of kaolin, but nevertheless, appreciably better than those grown in the cultures with quartz sand alone.

At the end of the third week, corn growing on quartz sand with 200 grams of kaolin was decidedly better than that growing on the quartz sand culture. The corn plants in the quartz sand cultures had a characteristic pale green color and the leaves were narrow. In the case where kaolin was added, the stalks were thicker, and the leaves decidedly darker in color. The corn in the quartz sand culture with 400 grams of Miami silt loam was not as good as in the case where kaolin was used. The leaves had reddish streaks indicating the presence of anthocyan, apparently showing a shortage of phosphorus. The weights of tops and roots are given in Table IV.

The data in the table indicate that the quartz sand culture with 400 grams of kaolin produced the highest weight of tops, as well as of roots. The yield from the quartz sand with Miami silt loam was considerably lower. However it is about the

same as from the quartz sand with 100 and 200 grams of kaolin. The quartz sand alone gave by far the lowest yield.

TABLE IV.

Effect on the growth of corn of the addition of kaolin and Miami silt loam to quartz sand cultures.

Pot No.	Treatment	Weight of dry tops in grams, ave. of two	Weight of dry roots in grams, ave. of two
1	Control	12·50	5·20
2	100 grams kaolin	17·00	6·31
3	200 grams kaolin	17·10	6·80
4	400 grams kaolin	21·50	8·30
5	400 grams Miami silt loam	17·00	5·75

It was thought that the beneficial effect of kaolin to plant growth might be due to certain elements found as impurities in kaolin, usually not considered essential. In order to remove the impurities from the kaolin, it was subjected to a treatment of 0·18 N HCl for 24 hours, washed until it gave no further test for chlorides, and then dried and put through a 100-mesh sieve.

Buckwheat was chosen for this next experiment because of its quick growth. The experiment was conducted in the same way as the previous ones. Two pots were provided with quartz sand and two with quartz sand *plus* 200 grams of washed kaolin.

The same general results were obtained with the washed kaolin as with the unwashed as is shown in Plate XXVI, fig. 2. The weights of oven-dried material are given in Table V.

TABLE V.

Effect on the growth of buckwheat of the addition of washed kaolin to quartz sand cultures.

Pot No.	Treatment	Weight of seeds in grams, ave. of two	Weight of dry tops in grams, ave. of two	Weight of dry roots in grams, ave. of two
1	Control	2·05	2·20	0·32
2	200 grams kaolin	2·60	2·80	0·60

Since the last traces of manganese could not be removed from kaolin by the acid washing, the following experiment was conducted in order to test the beneficial effect of certain elements usually considered unessential. The basal nutrient solution was shaken in one case with 20 grams of kaolin and in another with 20 grams of Miami silt loam for 12 hours. These solutions were filtered before using. In another set of pots, the distilled water used for watering was treated, in one case, with 400 grams of Miami silt loam and in another with 400 grams of kaolin. In this experiment there were seven different treatments, including the control, as indicated in Table VI, which also gives the yields.

TABLE VI.

Effect of kaolin and Miami silt loam on the growth of buckwheat in quartz sand culture.

Pot No.	Treatment	Weight of dry tops in grams, ave. of two	Weight of dry roots in grams, ave. of two
1	Control	4·15	0·35
2	Basal nutrient solution treated with 20 grams soil .	3·45	0·23
3	Basal nutrient solution treated with 20 grams kaolin	5·95	0·30
4	200 grams kaolin	8·27	0·75
5	500 grams of Miami silt loam	9·68	0·60
6	Distilled water for watering, treated with soil . .	3·85	0·23
7	Distilled water for watering, treated with kaolin .	6·16	0·55

As in the previous experiment with buckwheat, the quartz sand with kaolin and the quartz sand with soil produced the best growth. The plants of the control were drooping, spindling, and yellow in appearance. However, the plants in the quartz sand culture, the basal solution of which had been treated with soil, were even poorer than the control. The soil treatment decreased the supply of phosphorus to such an extent that it was the limiting factor. When the nutrient solution was treated with kaolin, some improvement was noticed over the control. The plants, however, were sub-normal, the leaves pale yellow, and the plants did not possess the rigidity of the normal plants. When the basal solution was made up with distilled water treated with soil the growth of buckwheat was the poorest,

though where kaolin was used, the growth was somewhat better than the control. Plate XXVI, fig. 3, gives the comparative growths.

Influence of other than 10 usual nutrient elements on plant growth.

Another experiment with buckwheat was conducted to study the effects of the addition of a number of the elements found in small amounts or traces in the ash of plants. These different elements were added along with the usual basal nutrient solution to quartz sand cultures in forms and amounts as follows :—

Boron as H_3BO_3, giving 0·5 mgm. B per culture
Zinc as $ZnSO_4$, giving 0·5 mgm. Zn per culture
Aluminum as $AlCl_3$, giving 0·5 mgm. Al per culture
Manganese as $MnSO_4$, giving 1·5 mgm. Mn per culture
Copper as $CuSO_4 \cdot 5\ H_2O$, giving 0·125 mgm. Cu per culture
Iodine as KI, giving 0·25 mgm. I per culture
Fluorine as NH_4Fl, giving 0·25 mgm. Fl per culture

The various treatments and results are given in Table VII. Lack of time and facilities did not allow the experiment to be carried out in greater detail so that all of the elements could be tested singly and in various combinations.

TABLE VII.

Growth of buckwheat produced in quartz sand cultures when additional elements are added to the basal nutrient solution.

Pot No.	Treatment	Weight of dry tops in grams, ave. of two	Weight of dry roots in grams, ave. of two
1	Control	2·64	0·29
2	B	3·05	0·36
3	Zn	2·98	0·35
4	B and Zn	3·65	0·39
5	B, Zn, and Al	3·75	0·42
6	B, Zn, Al, and Mn	5·40	0·55
7	B, Zn, Al, Mn, and Cu	4·00	0·40
8	B, Zn, Al, Mn, Cu and I	6·20	0·58
9	B, Zn, Al, Mn, Cu, I, and Fl	4·07	0·40

PLATE XXVII.

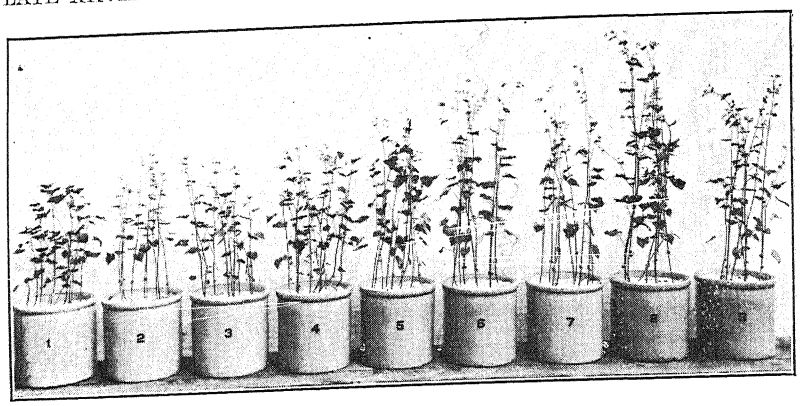


Fig. 1.—Effect of additional elements other than 10 essential elements on the growth of buckwheat. 1.—Control. 2.—Boron. 3.—Zinc. 4.—Boron and Zinc. 5.—Boron, zinc and aluminum. 6.—Boron, zinc, aluminum and manganese. 7.—Boron, zinc, aluminum, manganese and copper. 8.—Boron, zinc, aluminum, manganese, copper and iodine. 9.—Boron, zinc, aluminum, manganese, copper, iodine and fluorine.

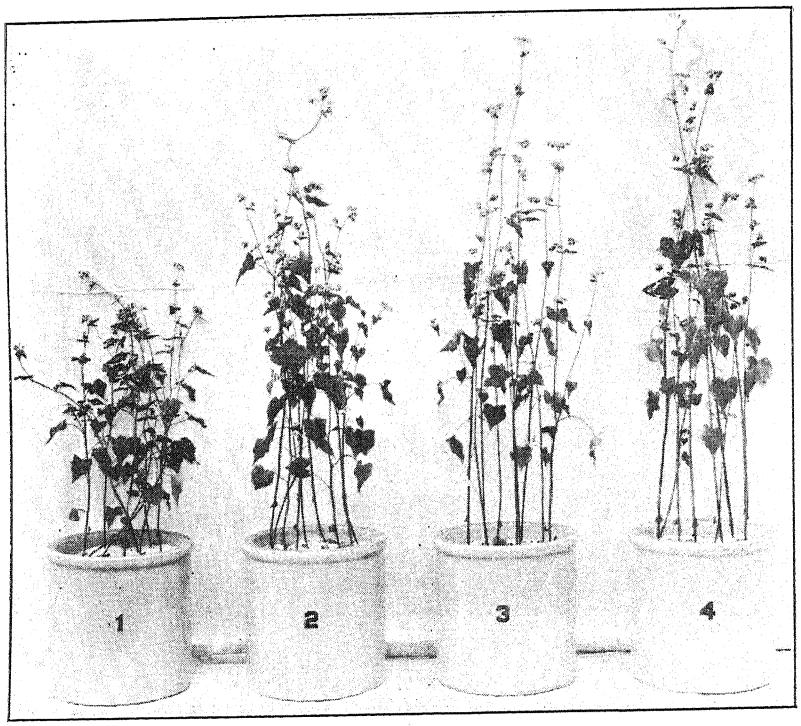


Fig. 2.—The growth of buckwheat in quartz sand cultures with complete nutrient solutions and other additions indicated. 1.—Control. 2.—200 grams kaolin and 20 grams calcium hydroxide. 3.—0·015 milligram manganous chloride. 4.—0·030 milligram manganous chloride.

In about 10 days, the plants in pots 5, 6, 7, 8 and 9 were ahead of those in the control. They were of a darker green color, thicker in stalk, and more rigid than the plants in the control. Plants in pots Nos. 2 and 3 were about the same as the plants in the control.

At time of harvesting, as indicated in Plate XXVII, fig. 1, the plants in all the treated pots were better than those in the control. The plants with the addition of boron alone, though taller, were spindling and not much better than those in the control. With the addition of zinc, the plants were not quite as tall as with the addition of boron, but were thicker in stalk. The boron and zinc combination was decidedly better than either alone. Aluminum was the first element in the series to produce marked beneficial results. The stalks of the buckwheat were thicker, the leaves larger, and darker green in color. With the addition of manganese, still further improvement in growth was noted. The plants were normal and characterized by a stiff, rigid stem structure. The addition of copper showed a detrimental effect. The plants were spindling and not normal in color. The application of iodine in addition to the others produced the most luxuriant growth. Fluorine offset the benefits of iodine and was apparently detrimental.

Manganese consistently gave increased yields. The marked increased yields of buckwheat due to iodine should be further tested before any definite conclusions are drawn. The effects of copper and fluorine appeared detrimental, at least in the amounts added.

The increased growth due to boron and zinc are not large enough to be very significent.

To obtain further evidence that manganese is essential for plant growth, buckwheat was grown in another set of cultures. Pot No. 1 contained quartz sand ; No. 2, quartz sand with 200 grams of kaolin impregnated with 20 grams of calcium hydroxide ; No. 3, quartz sand with 15 p. p. m. of manganese (as manganous chloride) added to the basal solution ; and No. 4, quartz sand with 30 p. p. m. of manganese. The kaolin was thoroughly mixed with the calcium hydroxide by standing in contact with excess of water for one day. Then carbon dioxide was passed into the solution until all the calcium hydroxide had changed to calcium carbonate.

All the plants germinated well and no differences appeared in growth until about the eighth day. Then the plants treated with manganese, kaolin, and calcium hydroxide surpassed the control. The stalks of the latter were thicker and more rigid and the leaves larger and darker green in color.

Somewhat later the plants treated with manganese slightly surpassed the plants in the quartz sand culture with kaolin and calcium hydroxide. The stems appeared somewhat thicker and the leaves larger and of a deeper green color. The

plants were also more rigid. However, the latter plants were no better than those obtained with kaolin alone. The slight depressing effect from the use of calcium hydroxide with kaolin is either due to neutralization of the slightly acidic property of the kaolin or to the fixation of manganese in difficultly soluble form. The weights of plants produced with these treatments are given in Table VIII, and the general appearance is shown in Plate XXVII, fig. 2.

The roots produced where manganese was added were as extensive as where kaolin was added.

TABLE VIII.

Effects of manganese on the growth of buckwheat in quartz sand cultures.

Pot No.	Treatment	Weight of dry tops in grams, ave. of two	Weight of dry roots in grams, ave. of two
1	Control	4·54	0·20
2	200 grams kaolin *plus* 20 grams calcium hydroxide . .	8·10	0·76
3	0·015 gram manganous chloride	8·15	0·67
4	0·030 gram manganous chloride	9·46	0·75

Influence of limestone with kaolin on plant growth.

In another experiment, crushed limestone was employed to see whether it would decrease the growth of buckwheat by neutralizing the acidic property of kaolin. In this experiments, alfalfa and buckwheat were grown. The following treatments were provided in duplicate, *viz.*, quartz sand, quartz sand and 16 grams of crushed limestone, and quartz sand with 200 grams of kaolin and 16 grams of crushed limestone. To all of them the regular amount of basal nutrient solution was added.

In about 5 days, the leaves of the alfalfa with the quartz sand and kaolin had a deeper green color and were also broader. The difference in growth became greater as time went on. With the crushed limestone treatment there was no improvement over the control. At the time of harvest it was noticed that the nodules were plentiful on the roots of alfalfa where kaolin was used, but there were none present where quartz sand or crushed limestone was employed. Since the crop was not inoculated, the bacteria were undoubtedly carried by the seed. Table IX gives the weights of the tops and roots.

TABLE IX.

Effect of the addition of limestone and kaolin on the growth of alfalfa.

Treatment	Weights of dry tops in grams, ave. of two	Weights of dry roots in grams, ave. of two
Control	2·00	1·25
16 grams crushed limestone	1·50	0·75
200 grams kaolin and 16 grams crushed limestone	4·06	2·50

With the buckwheat similar results were obtained as in the case of alfalfa, the crushed limestone did not depress the growth of the buckwheat grown on the quartz sand culture with kaolin. The plants were dark green and rigid, with long internodes. The control plants were spindling and of light green color. Pot No. 2, with the addition of crushed limestone to the quartz sand, was somewhat better than the control, but not nearly as good as the one with kaolin. The weights of the tops and roots are given in Table X.

TABLE X.

Effect of the addition of limestone on the growth of buckwheat.

Pot No.	Treatment	Weight of dry tops in grams, ave. of two	Weight of dry roots in gram ave. of two
1	Control	3·3	0·27
2	16 grams crushed limestone	5·75	0·42
3	200 grams kaolin and 16 grams crushed limestone	9·05	0·78

Combined influence of kaolin and manganese on plant growth.

To ascertain whether the addition of manganese would produce as good a crop as the addition of kaolin and also whether the addition of manganese with the kaolin is advantageous, buckwheat was grown in pots of 1-gallon capacity. One-half the amount of quartz sand as well as of the basal nutrient solution was used as with the 2-gallon pots. To pot No. 1, 0·015 gram of manganous chloride was added; and to pot No. 2, 100 grams of kaolin and 0·015 gram of manganous chloride.

For the first two weeks, there were no significant differences in height with the various treatments. Later, the plants with kaolin alone pushed slightly ahead of

those with kaolin and manganese, and also those with manganese alone. The plants with the kaolin treatment were somewhat thicker in stalk and the leaves somewhat larger and darker green in color than those with manganese alone. The experiment indicates that manganese added to the regular basal solution has provided conditions favorable for normal growth, and a medium nearly as good as when kaolin is added. It also indicates that kaolin may carry sufficient manganese as an impurity so that a further addition above that carried by it produces no response. The results of the experiment suggest that kaolin has some additional beneficial effect on plant growth aside from supplying manganese.

TABLE XI.

Effect of manganese and kaolin on quartz sand culture of buckwheat.

Pot No.	Treatment	Weight of dry tops in grams, ave. of two	Weight of roots in grams, ave. of two
1	0·015 gram manganese	3·28	0·422
2	100 grams kaolin	3·97	0·430
3	100 grams kaolin and 0·015 gram manganous chloride .	3·54	0·427

Table XI gives the weights of plant tissue produced. There is no significant difference in weight of roots produced.

No experimental work was done to study the effect of the colloidal property of kaolin on plant growth in quartz sand cultures. From work done in this field it seems highly improbable that the beneficial effect of kaolin is due to physical influences.

DISCUSSION.

The evidence obtained in the experiments indicates that some important soil factors are often neglected in growing plants in a synthetic culture medium. The foregoing experimental evidence, as well as that of McHargue and Sommer and of Lipman and others, indicates that normal plants cannot be produced in a culture medium which contains only the usual 10 elements.

From the results obtained in the preceding experiments it is evident that marked improvements in plant growth may be obtained with manganese. What role it plays in the nutrition of the plant is not known. McHargue has assigned the role of a catalyst to manganese, and thinks it is connected with the formation of chlorophyl. Others maintain that it acts as an oxidizing agent in the soil

solution, destroying toxic organic material. The writer noticed that the leaves were darker in color and the roots better developed where manganese was added to the regular basal solution. Where kaolin was added, it is probable that the deficiency of manganese was met to a certain extent. Even in the washed kaolin there were 20 p. p. m. of manganese.

Iodine gave marked beneficial effects on plant growth in quartz sand cultures. The number of cultures employed was not sufficient to allow the drawing of final conclusions as regards iodine. Aluminum also gave beneficial effects, and boron and zinc to a lesser extent. Copper and fluorine did not improve the growth of crops in quartz sand cultures.

From the work presented it appears that kaolin has a two-fold effect on the growth of plants. Judging from the marked improved root development with kaolin it seems that it has a very beneficial effect on the roots. It is possible that toxic effects from the decomposition of root hairs and root caps may be lessened or prevented by kaolin. If the toxins were basic in character, they could be taken up by the kaolin, since it is usually slightly acidic and in this way the amount of toxin in solution at any one time would be greatly lessened. In other words, the kaolin might act as a buffer against toxic material.

SUMMARY.

The object of this study was to investigate some of the neglected soil factors in plant growth. It was observed in preliminary tests that when kaolin was added to the usual sand cultures better plant growth was obtained. As regards the factors involved, there appeared to be at least three possibilities, *viz.*, (*a*) that kaolin contains as impurities small amounts of elements other than the usual 10 which stimulate plant growth; (*b*) that the acidic property of kaolin in some manner benefits plant growth, possibly by combining with toxic material of a basic nature; and (*c*) that the colloidal property affects plant growth through physical means. Only the first two factors were studied.

Synthetic sand cultures with basal nutrient solutions, containing the usual 10 nutrient elements were used. The work was carried on in the greenhouse under controlled conditions.

For the study of the influence of the addition of kaolin and soil to quartz sand cultures with the regular basal solutions, alfalfa, buckwheat, oats, and corn were chosen as test crops. With these crops, the kaolin materially increased the growth of the tops and usually also that of the roots. The plants in the control cultures were often spindling and pale green in color, while those grown in the quartz sand with kaolin had a thick rigid stem and were dark green in color. The leaves were also considerably larger in size. Miami silt loam had a favorable

effect like kaolin only in the case of buckwheat. With the other crops fixation of phosphate by the soil may have made phosphorus a limiting factor.

In a test with buckwheat, acid-washed kaolin gave the same results as the unwashed material. There still remained some 20 p. p. m. of manganese in this kaolin.

Treatment of the basal nutrient solution with soil and kaolin was detrimental in the case of soil but somewhat beneficial in the case of kaolin. Treatment of the distilled water used for watering with soil and kaolin was also detrimental in the case of soil and beneficial in the case of kaolin.

Boron, zinc, aluminum, manganese, copper, iodine and fluorine were the additional elements added to quartz sand cultures besides those in the regular basal solution. Manganese consistently gave favorable results with buckwheat. Boron, zinc, aluminum, and iodine seemed beneficial, whereas copper and fluorine gave no apparent increased growth.

The use of calcium hydroxide with kaolin had a slight depressing effect on the growth of buckwheat compared to kaolin alone, apparently due to the neutralization of the slightly acidic property of kaolin. However, when crushed limestone was used instead of calcium hydroxide, no depressing effects were observed.

Kaolin produced somewhat more benefit than manganese. The addition of manganese with kaolin produced no more benefit than kaolin alone. The results indicate that kaolin has some additional beneficial effect on plant growth aside from supplying manganese.

The investigation shows that kaolin, as ordinarily found, is beneficial to plant growth. Impurities in the kaolin, such as manganese, seem to account partially for the benefit. Whether or not the kaolin carries manganese in an especially suitable form for plants is not known. The slightly acidic nature of kaolin may account partially for its beneficial effect.

The results of this investigation support those of others that manganese and iodine are essential for the best plant growth.

LITERATURE CITED.

1. Bertrand, Gabriel. Manganese as a catalytic fertilizer. Paris Org. Com. 8th Intern. Congress Appl. Chem. 15 : 39. 1910.
2. ——— Sur le role dis infiniment petits chiminques en agriculture. Ann. Inst. Pasteur, I 26, II : 852-867, 1 Fig. 1912.
3. Breazeale, J. F. Effect of certain solids upon the growth of seedlings in water cultures. Bot. Gaz., 41 : 54—63. 1906.
4. Brenchley, W. E. The influence of copper sulphate and of manganese upon the growth of barley. Roth. Exp. Sta. Ann. Rep., 7 ; Ann. Bot., 24 : 571—583. 1910.
5. ——— Inorganic plant poisons and stimulants. Cambridge Univ. Press, 1—10: Pl. 7, Fig. 12. 1914.

6. Contino, A. Amount of manganese in some Italian soils. Staz. Sper. Agrar. Ital., 44: 51—55; Jour. Chem. Soc. London, Aii, 649. 1914.
7. Dandeno, J. B. The relation of mass action and physical affinity to toxicity with incidental discussion as to how far electrolytic dissociation may be involved. Amer. Jour. Sci., 17: 437—458. 1904.
8. Funchess, M. J. The effects of certain organic compounds on plant growth. Ala. Agr. Exp. Sta. Bul. 191. 1916.
9. Hiltner, L., and Korff, G. The effect of various soil treatments, especially of manganese sulphate upon the growth of oats. Prakt. Blatt. Pflanzenbau U Pflanzenschultz, 15: 549—556. 1918.
10. Jardin, F., and Astruc, A. Arsenic and manganese in leaves at different stages. Compt. rend. Acad. Sci. (Paris), 156: 2023—2024. 1913; Exp. Sta. Rec., 29: 628. 1913.
11. Jensen, G. H. Toxic limits and stimulation effects of some salts and poisons on wheat. Bot. Gaz., 43: 11—44. 1907.
12. Livingston, B. E. Further studies on the properties of unproductive soils. U. S. D. A. Bur. Soils Bul. 36: 71. 1907.
13. Maze, P. Influences respectives des elements de la solution minerals sur le developpement du mois. Ann. Inst. Pasteur, 28: 21—68. 1914.
14. McHargue, J. S. Occurrence and significance of manganese in the seed of various plants. Jour. Amer. Chem. Soc. 36: 2532—2536. 1914.
15. ———— Manganese and plant growth. Jour. Ind. and Eng. Chem., 18: 172—175. 1926.
16. ————, and Shedd, O. M. The effect of manganese, copper, zinc, boron, and arsenic on the growth of oats. Jour. Amer. Soc. Agron., 22: 739—746. 1930.
17. McLean, F. T., and Gilbert, B. E. Manganese as a cure for a chlorosis of spinach. Science, 61: 636—637. 1925.
18. Nageli, C. Ueber oligodynamische Erscheinuagen in lebenden Zellen. Denkschr. Schweiz. Naturf. Bes., 33: 1—43. 1893.
19. Shedd, O. M. The occurrence of manganese in Kentucky soils and its possible significance. Jour. Ind. and Eng. Chem., 6: 660. 1914; Jour. Amer. Chem. Soc., 36: 2532. 1914.
20. Skinner, J. J., and Reed, F. R. The action of manganese under acid and neutral soil conditions. U. S. D. A. Bur. Plant Ind. Bul. 441: 12. 1916.
21. Sommer, A. L., and Lipman, C. B. Evidence on the indispensable nature of zinc and boron for higher green plants. Plant Physiology, 1: 231—247. 1926.
22. Ture, R. H., and Oglevee, C. S. The effect of the presence of insoluble substances on the toxic actions of poisons. Bot. Gaz., 39: 1—21. 1905.
23. Truog, E., and Sykora, J. Soil constituents which inhibit the action of plant toxins. Soil Science, 3: 333—352. 1917.

THE ACTION OF TOXIC AGENTS USED IN THE ERADICATION OF NOXIOUS PLANTS[1]

BY

R. B. HARVEY[2]

(With Plates XXVIII & XXIX.)

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The substances now used for killing noxious weeds and shrubs may be classified on the basis of their type of toxic action as follows: First, substances which by their osmotic action plasmolyze cells and prevent plants which are treated with them from obtaining water. An example of such substances is common salt. A second class includes those substances, such as hydrocarbons, which by their physical action dissolve or dilute protoplasmic constituents and disorganize the cell by changing its permeability and other physical properties. A third type includes the protoplasmic poisons which stop the action of enzymes, coagulate protein, or combine with other constituents of the protoplasm. Examples of this class are mercuric chloride, cyanides, copper salts, ferrous sulphate, etc. Recent work of the author indicates that this is a type of action exhibited by certain substances which react with the respiratory pigments of plants and interfere with the oxidation-reduction balance in cells. An example of this class is sulfur dioxide, which causes the reduction of respiratory pigments so that they can no longer function. Other agents of this type will be discussed later. All of these types may be shown by a substance, the main action being that which comes to expression at the lowest concentrations. For instance, mercuric chloride has an osmotic action, but this does not come into effect generally because the effect of the mercuric ion as a coagulant of proteins occurs at much lower concentrations.

To use the osmotic action for killing plants requires that the concentration of the solution shall be rather high, at least higher than the osmotic concentration of the cells of the plant to be killed. The usual range of osmotic pressure in plants is from 5 to 20 atmospheres, but osmotic concentrations of some halophytes may be as high as 161 atmospheres. Dry seeds may be able to imbibe water against a force

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of nearly 1,000 atmospheres of osmotic pressure. Large quantities of the plasmolyzing agent are required to kill, and to be useful for an eradicant the substance must be very inexpensive.

The use of pure saturated hydrocarbons, gasoline, or oils to eliminate plant pests is dependent upon penetration and conduction into the plant in concentrations sufficient to kill. The plant cell is principally water, and to have more than a local action the hydrocarbons must either be soluble in water or penetrate in the gaseous condition.

Of the protoplasmic poisons, those which coagulate proteins, such as salts of the heavy metals, are quite effective for use on thin layers of tissues, such as leaves, but where masses of tissue are concerned their action as protein coagulants prevents their penetration deep into the tissue. A considerable quantity of salts of the heavy metals is required because the metallic ions are combined with and precipitated by the proteins. Also, salts of the heavy metals are held by the soil and may have a lasting effect on soil fertility.

The substances which act by disarrangement of the oxidation-reduction system of cells seem to be of two types. First are those, like sulfur dioxide, which are strongly reducing subtances which reduce the oxygen acceptors of tissues so that they are unable to combine with oxygen. Tissues treated with sulfur dioxide do not turn darker on exposure to air. The respiratory chromogens evidently are reduced to such a state that they no longer serve as carriers of oxygen. The other type of action is that shown by chlorates and ethylene oxide. The tissues turn black and the cells die. Evidently the respiratory chromogens are so completely oxidized that they cannot function. They are oxidized to the pigment forms peculiar to each tissue. Thus, the leaves, bark, and young woody tissues of popple turn black after treatment with solutions of ethylene oxide and of sodium chlorate. The tissues quickly die, and the extent of the killing can be judged by the blackening of the tissues.

The quantity of respiratory chromogens present in cells is not great, so the quantity of toxic agent required to upset the oxidation-reduction conditions of the cell is not great. The use of chlorates on Canadian thistle (*Circium Arvense*) has shown the blackening of leaves and stems within 48 hours after spraying chlorates upon them. The roots of leafy spurge (*Euphorbia esula*) turn black on treatment with ethylene oxide.

In 1924, during the investigations of this laboratory on the ripening of fruits and vegetables and the blanching of celery by means of ethylene gas, ethylene oxide was tried as a ripening agent. On September 10, bananas treated with ethylene oxide, 1 part in 1,000 parts of air, did not ripen but turned black very quickly. The skins were very black in 24 hours after treatment, and the flesh was blackened

along the vascular tracts. The use of ethylene oxide as a ripening agent was abandoned. In October, 1924, trial was made of the toxic effects of ethylene oxide on animals. A rooster was placed in an atmosphere containing ethylene oxide, and the concentration was gradually increased in the chamber until the rooster seemed to be anaesthetized. Then he was brought out into open air. His comb turned very dark in color and he died. The toxic effect of ethylene oxide was evident.

Ethylene oxide was tried also for its use in breaking the dormancy of potato tubers. All tubers treated with ethylene oxide turned black and the tissue was killed. The use of ethylene oxide to break dormancy was abandoned [1].

Among a large number of substances which were tried for their effectiveness in the eradication of noxious weed, it was decided to include ethylene oxide. On December 10, 1929, a pan of quack grass was treated with a 10 per cent. solution of ethylene oxide in water, using 300 cc of this dilution in a galvanized iron pan 10 inches wide, 12 inches long, and 4 inches deep, filled with quack grass sod. The rhizomes were all killed, but on February 10 it was noticed that quack grass seed and weed seeds had sprouted in the same soil. This indicated that ethylene oxide was an effective killing agent, but that its effect was not lasting in the treated soil.

On April 19, 1930, barberry bushes growing on the grounds of the State Agricultural Society were treated with ethylene oxide, pouring liquid from a graduated cylinder into hole made beneath the bush. One bush with eight stems about 3 feet high was given 30 cc of ethylene oxide. The crown of this bush was 6 inches from a Crataegus tree. On June 18 all but two sprouts nearest the Crataegus were dead, while the Crataegus tree was uninjured. A smaller bush with four shoots 2 feet high was also given 30 cc on April 19. A Crataegus tree was 1 foot away from the bush. On June 18 both the barberry and the Crataegus were killed. Evidently the placing of the charge determined the range of the killing area.

Various devices were then tried for injecting the ethylene oxide into the soil and for measuring the dose. On August 12 a group of 16 large barberries was found at Afton, Minnesota, and was treated with ethylene oxide and diethylene oxide, measuring different doses into holes made by a rod under the bushes. On one bush in this group, which was given 0·4 kilo of a mixture of 500 cc of ethylene oxide with 4,500 cc of water, only the young stems and some leaves were dead and blackened August 19. On September 17 all of the leaves on this plant were dead except those on a single branch opposite the point of injection of the ethylene oxide. These were browned and reddened along the veins, evidently due to the production of anthocyanins. A second bush showed some green leaves on one side. Seven other bushes in this group were all dead on September 17. The cambium and inner

[1] This was reported in a paper by Bacha and Harvey in Plant Physiology, 2; 187—193, 1927.

PLATE XXIX.

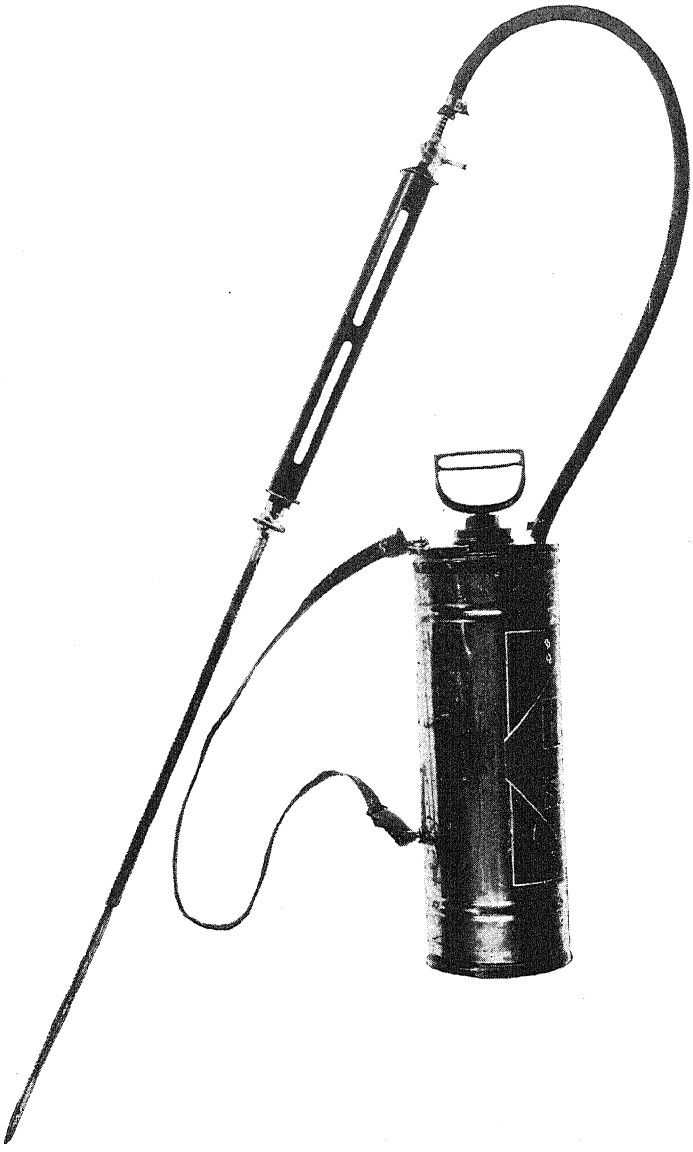


Fig. 1.—"Gopher stick" on a knap sack sprayer.

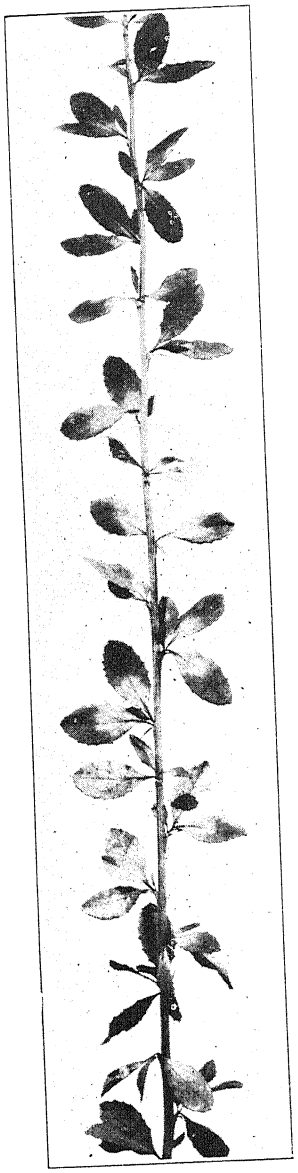


Fig. 2.—Barberry short, showing blackening of leaves 8 days after treating with ethylene oxide.

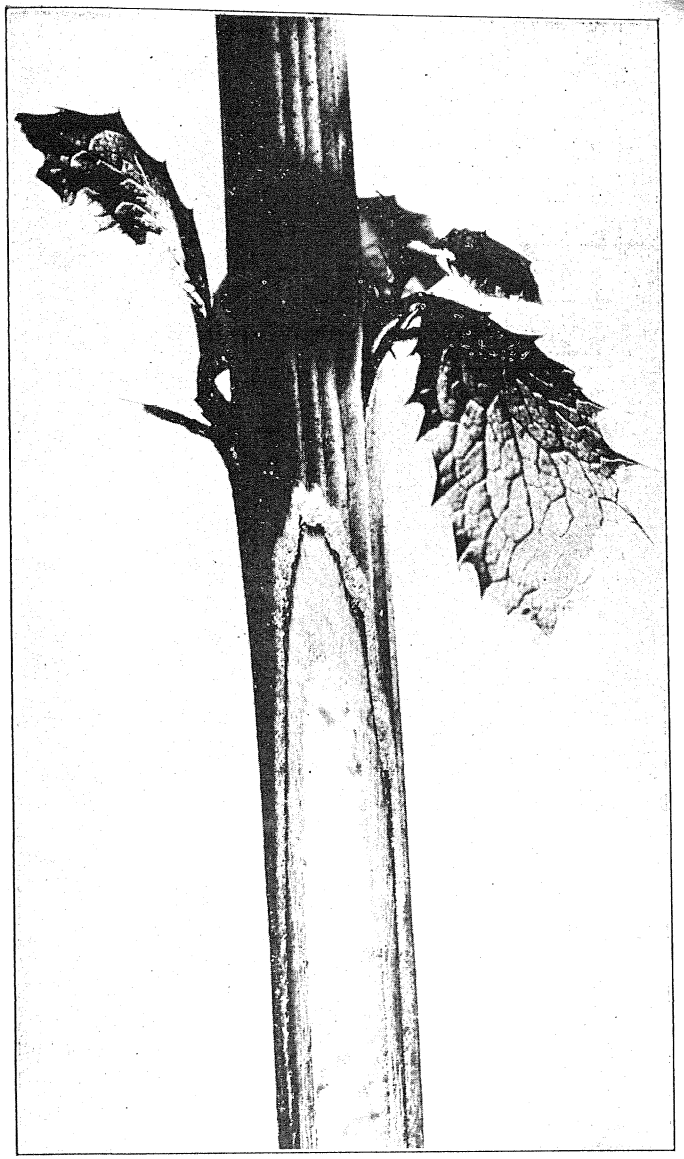


Fig. 1.—Barberry branch, showing blackening of the cambium layer 8 days after treating with ethylene oxide.

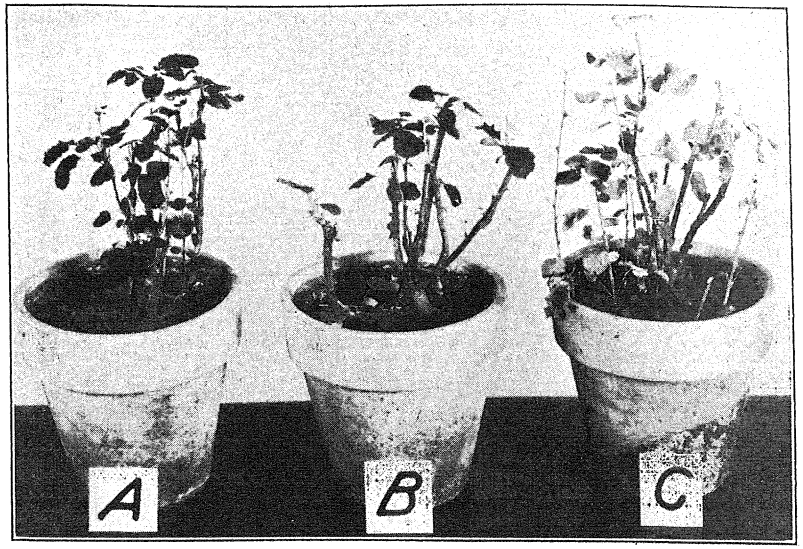


Fig. 2.—Potted barberries treated with propylene oxide. A.—0·1 cc propylene oxide, no apparent injury. B.—1·0 cc propylene oxide, some injury on leaves. C.—10·0 cc propylene oxide, plant dead.

bark and younger wood were blackened, as shown in Plate XXVIII, fig. 1. The leaves also blacken when the dose is large enough to kill (Plate XXIX, fig. 2).

The barberry eradication campaign and the campaigns for the control of white pine blister rust through the eradication of currants and gooseberries have shown the need of a chemical substance with high toxicity to the noxious plants yet which will have no lasting detrimental effect on the soil. Ethylene oxide seems to be such a substance. The practice of digging out barberry bushes leaves a possibility of sprouts being produced from pieces of roots not removed in the digging. The use of common salt is a more desirable practice from the standpoint of the labor involved and the effectiveness of the killing agent. For use in pastures this method may have some objection. The use of chlorates, arsenates, etc., is excluded in pastures where cattle may be poisoned.

By the use of a rod, ethylene oxide can be introduced into the soil beneath bushes or into layers of soil below the plow sole. A "depth charge" can be regulated to certain levels of roots in the soil. The materials so injected are not accessible to animals. Ethylene oxide is liquid at ordinary temperatures at pressures between 8 and 20 pounds per square inch. This gives pressure sufficient to drive it into the soil directly from the tank. A special measuring device fitted to an injecting rod has been devised, which has been called a "gopher stick".

The ethylene oxide is volatile enough to allow a quick spread through the soil. A relatively short period of its effect in the soil is indicated by results so far obtained. It is soluble in water, and dilutions with ice cold water can be made with little loss when it is desired to use a water dilution or a mixture with other toxic agents. Dilutions can be handled in the usual knapsack sprayer, with a "gopher stick" (Plate XXIX, fig. 1) in place of the spray nozzle. Mixtures with chlorates or formaldehyde can be used without chemical reaction destroying the toxicity.

The use of ethylene oxide alone and in water solution to date has been shown by the killing of several hundred bushes of barberry, currant, gooseberry, poison ivy, prickly ash, scrub oaks, popple, boxelder, etc. The size of the charge of dose must be adjusted to the bush to be eradicated. Determinations have been made on the charge required in different types of soils and with various soil moisture contents. Indications are that at the present price of ethylene oxide the cost of materials is about the same as for eradication by common salt, while the labor is considerably reduced.

The practical applications of ethylene oxide under various conditions have shown that the action can be localized so as to kill roots which lie deep in the soil without killing plants whose roots are shallow. By placing a depth charge of the ethylene oxide 18 inches below the surface near roots of barberry, it has been

possible to kill the bushes without killing grass around them. There may be some disadvantage in not killing seedling barberries just starting beneath the bushes.

For shallow-rooted shrubs, such as hazel bush, it is desirable to put the charge beneath the mat of roots to decrease the loss of the ethylene oxide from the soil. Perhaps ethylene oxide will not prove effective as an eradicating agent for shallow-rooted weeds, such as quack grass, on account of its rapid diffusion from the soil. When sprinkled in water dilutions onto quack grass sod, little killing effect is shown. Higher members of the oxides of unsaturated hydrocarbons, such as propylene and butylene oxides, have a decreased volatility and may be more useful for this purpose.

Diethylene oxide is less volatile than ethylene oxide, but comparative tests run under the same conditions show the diethylene oxide to be not injurious to plants treated with quantities such as were found lethal in the use of ethylene oxide.

Propylene oxide (B. P. 35°C) is less volatile than ethylene oxide (B. P. 10·5°C). This may decrease its penetration through the soil as a gas. Propylene oxide is soluble in water and may move with the soil water and be absorbed by the roots. Propylene oxide has been applied to potted barberries and other plants and on a few plants in the field. In the field trials evidently the concentrations were too low to give observable effects, but in the greenhouse pot experiments the toxic doses show injury of the same nature as ethylene oxide and at approximately the same doses. Plate XXVIII, fig. 2 shows the effect of graduated doses of propylene oxide in killing barberries. The tissues of the cambium are blackened and the leaves show reddening or blackening and then drop off much as in the treatment with ethylene oxide.

Flats 18 × 16·4 inches were filled with moist soil, and to each was added 100 cc of ethylene oxide or propylene oxide dissolved in water. Dry seeds of wheat, peas, radish, corn, and oats were planted in these flats on the same day that oxides were applied and on the second, third, and fourth days after. Seeds planted on the first day were nearly all killed by both ethylene oxide and propylene oxide. There was injury to those planted on the second and third day after treatment, but those planted on the fourth day gave normal seedlings. On account of the shallowness of the layer of soil, it will be necessary to try out the treatment under field conditions with various crops before conclusive data can be obtained, but the indications are that the effect of the oxides of the unsaturated hydrocarbons is much less lasting than the effects of chlorates.

These oxides of unsaturated hydrocarbons may be manufactured from natural gases or from gases produced in the cracking of petroleum. These gases are waste products in some regions. The process of manufacture is relatively simple, being

first a chlorination of the unsaturated hydrocarbons in water to produce ethylene or propylene chlorhydrin. On treatment with alkali, the chlorhydrin yields the corresponding oxide. The chlorine and alkali are easily produced from common salt by electrolysis. The availability of cheap electrical power, as at Muscle Shoals, near a supply of waste unsaturated hydrocarbons, as in West Virginia, should make these oxides inexpensive. Their toxicity in low concentrations seems to open up possibilities for them in the general eradication of the more noxious deep-rooted weeds. The ease of handling these oxides is much greater than for common salt or the chlorates. This is of especial advantage in woods or rough country where the materials must be carried. A man can carry easily the 17-pound cylinder (total weight, 40 pounds) in an army pack sack. This is enough to treat more than a hundred bushes of barberry or gooseberry.

The action of these oxides seems to be upon the respiratory mechanism of cells. Treatment of tissues with ethylene or propylene oxide decreases catalase and oxidase activity. The blackening of the tissues is evidence of the oxidation of the respiratory chromogens.

SUMMARY.

Ethylene and propylene oxides were found to be useful eradicants for noxious plants. They offer advantages over present methods in ease of handling, in toxicity in small concentrations, and in rapid release from the soil, thereby decreasing the time of unproductivity in comparison with chlorates and chlorides.

ABSTRACTS

Forest soil and vegetation in the Hlaing Forest Circle, Burma. A. H. M. Barrington. (*Burma Forest Bulletin No. 25.*)

Fifty-three plots were chosen for experimental purposes, the size of plots being determined by the height of trees growing on them. A botanical census was made and soil samples analysed. The object of the investigation was to explain the distribution and range of growth of the more important trees in relation to the soil on which they occur, since planting must now be carried out on soils other than those which grew the same species well previously. Also there is evidence from both Madras and Burma that teak does not grow well on old teak plantations.

The limits of the Hlaing Circle fall between Latitude 16° 20′—19° 50′ and Longitude 95° 10′—96°25′. Rainfall varies from about 30 in. per annum in the north to over 100 in. per annum in the south. The area of reserved forest is 2,043 square miles. Plantations amount to 103 square miles and annually increase by more than 2 square miles.

From the mechanical analysis figures, utilizing the higher size limits, was calculated a figure called the *texture index number* and from the sticky point moisture determinations a round figure of 16 per cent. was deducted, the remaining moisture being regarded as *colloidally held water*. Classification was chiefly on a space diagram from these determinations but in addition the distribution of species appears to be related to the exchangeable base content of the soils.

Best forest growth always occurs on new or immature soils as soils deteriorate as they mature. Indaing (*Dipterocarpetum tuberculati*) soils exhibit some characteristics of a podsol while it appears that in this area the lateritic soils only approach lateritites.

The upper 3″-4″ of soil determines the predominance of Indaing which grows on the lightest soils irrespective of rainfall. Regeneration ceases if the light surface soil is lost or if floods deposit clay on the surface and Indaing may therefore be left stranded on a non-typical soil if it has become established before this occurs Usually these soils are faintly acid.

Teak is dominant on soils closely allied to Indaing and frequently occurs growing well on soils devoid of free carbonates.

Ingyin (Pentacme suavis) soils form a compact group in which the texture index usually diminishes with depth. In calcareous scrub the replaceable bases are excessively high (under light rainfall).

Teak on medium soils appears to grow excellently without free carbonates while its presence in laterites also shows its independence of carbonates. It is probably only on the stiffer soils where carbonates are necessary.

Xylia laterite is common north of Rangoon and appears to be derived from Rangoon lateritite by erosion.

The best teak soils have the relationship:

Texture Index No. $=3{\cdot}6+(0{\cdot}5\times$ Colloidally held $H_2O)$. Planting of teak (if policy permits) should be governed by the following considerations:—

(1) Teak will not survive to maturity on heavy soils unless these are calcareous.

(2) First rate growth may be expected in good kyathaung (Bambusa polymorpha) forest, on well drained alluvium where the texture is not too heavy and on heavy calcareous soils in dry mixed forest.

(3) Second or third class plantations can be formed in dry mixed forest on the lighter soils whether these are calcareous or not.

(4) Third rate plantations can be formed on Rangoon lateritite, alluvial sands derived from Indaing soils and local deposits of clay over sand.

(5) Only stunted teak can be expected on Indaing and Ingyin soils on the one hand and heavy calcareous scrub soils on the other.

Pyinkado (Xylia dolabriformis) has often been planted on soils considered too heavy for teak. There is reason to think this is a mistake. Pyinkado appears to avoid calcareous soils. It is likely to grow as well as teak on Rangoon lateritite soils but its real habitat is in the climax moist deciduous forest.

Taukkyan (Terminalia tomentosa) is dominant on calcareous scrub, on one dry deciduous medium, on one alluvial lower mixed, on one Ingyin and on three moist deciduous forest soils. It is a wide-spread species but is probably excluded from the best soils by teak and pyinkado. It is characteristic of heavy soils, calcareous or not but grows after a fashion on Indaing sands and dry dipterocarp soils generally. There is no question of planting at present values. [J. C.]

A Cytological Study of the Genus Sorghum *Pers.* 1. The Somatic Chromosomes.

C. Leonard Huskins and Stanley G. Smith.

[*Journal of Genetics*, Vol. 25, No. 2, February 1932.]

This study was undertaken at the request of the Director of the Royal Botanical Gardens, Kew, and was intended as a preliminary to the formulation of certain economic breeding plans and as a possible corollary to Dr. Stapf's system of classification. The somatic chromosome number of Johnson grass, *S. halepense* is 40. Five wild sorghums, *viz.*, *S. virgatum*, *S. verticiliflorum*, *S. vogelianum*, *S. lanceolatum*, *S. arundinaceum* and the cultivated grass *S. sudanense*, all have 20 chromosomes as have the fifteen "grain" sorghums examined. One peculiar-shaped chromosome can be identified in all the species; it is present in duplicate in *S. halepense* only. Thus the cytological observations are in accord with the discovery by Vinall [1926] that crosses between Johnson grass and the annual sorghum can be made only with great difficulty, yielding almost sterile progeny, whereas the wild annual sorghums cross freely with the large annual grain sorghums. The morphological differences in the chromosome sets of the different sub-species of cultivated sorghums, though small, appear to be sufficiently definite for purposes of classification. Chromosome doubling occurs in the root tissues and, if occurs or can be caused to occur in the stem, it should be possible to obtain hybrid polyploid strains for economic purposes. [B. C. B.]

NOTES.

NEW INTERNATIONAL SYMBOLS FOR THE MAPPING OF LOCUSTS.

" The International Institute of Agriculture, Rome, has issued the proceedings of an international conference held at Rome on 28th September to 1st October 1931, for the study of the migratory locust problem. The Conference which was purely scientific and technical recommended the adoption of a uniform set of symbols to facilitate the mapping of locust movements ; it further recommended that all locust reports should be accompanied by maps and that the following symbols should be used.

1. Flying swarms in a known direction, date and approximate dimensions to be shown on the sides of the arrow. ↑
2. Flying swarms direction not reported . . . . . T
3. A stationary swarm, *i.e.*, a swarm that has alighted . . . . ▲
4. A swarm which alighted and subsequently left in a known direction . ▲|
5. A swarm which came *from* a known direction and alighted . . . ↑▲
6. A disturbed flying swarm (circling) . . . . . 
7. A swarm which lays . . . . . △
8. Eggs laid (place) . . . . . o
9. Larvæ . . . . . Number stages where possible . ●

Typical solitary phase . . . . . s

Colours to be used for fliers only.

A. Rose-coloured adults	Red	↑ or T
B. Brown—Red ,, ...	Green	
C. Yellow adults	Blue	
Colour not reported	Black	

THE INTERNATIONAL YEAR BOOK OF AGRICULTURAL STATISTICS.

The International Institute of Agriculture at Rome has recently published the 1930-31 edition of the "International Yearbook of Agricultural Statistics".

This volume of 830 pages is the result of the most extensive and detailed inquiry made in the domain of international agricultural statistics and constitutes a work of the greatest importance to all those who are interested in questions having a direct or indirect relation to production and commerce of agricultural products.

In the first part of the Yearbook are classified the figures for area and population in the years nearest to 1913 and 1930 for 220 countries: the presentation of these figures throws light upon the world situation from the geographical, political and demographical points of view during both the pre-war and post-war periods. The second part is composed of a series of tables comprising for nearly 50 countries the available data concerning the uses for which the total area is employed, the appointment of cultivated areas between the different crops, agricultural production, numbers of the different kinds of livestock and the products derived from them. In the tables constituting the third part of the volume, have been indicated for nearly 40 agricultural products, the area, production and yield per acre in each country during the last five years of the pre-war period and during each of the years from 1927 to 1930.

For each kind of livestock all available figures in the different countries have been grouped for the years 1913 and 1926 to 1930. A large part of the volume is devoted to statistics of the commercial movement of 42 vegetable products and 12 products of animal origin. The figures published relate to the imports and exports during the calendar years and for the cereals also during the commercial seasons.

It may be added that the tables of production and commerce not only specify details for each country but also the totals for the different continents and hemispheres and for the whole world, allowing the formation of a general idea of the changes taking place during the periods under consideration in the area under each crop, quantities harvested and the commercial movement in each product.

The part devoted to prices contains the weekly quotations of 25 agricultural products on the principal world markets for the year 1913 and for the period January 1927 to July 1931. In the freights section will be found the quotations for the transport of wheat, maize and rice on the most important shipping routes, and in the section reserved for fertilizers and chemical products useful in

agriculture are published statistics of production, trade and prices for 15 products. In the rates of exchange section are set out the rates on the New York exchange for the most important currencies and in the Appendix have been brought together special chapters on the importance and distribution of the agricultural population, the distribution of agricultural holdings according to their size and mode of tenure and forestry.

The volume has also been enriched by a long introduction and a chapter of explanatory notes.

THE IMPERIAL BUREAU OF SOIL SCIENCE.

The Imperial Bureau of Soil Science issues two regular sets of publications, *viz*:—(1) "Technical Communications" (printed or duplicated) dealing with some particular aspect of soil science; about six of these are issued annually the prices ranging from 6d. to 2s. (2) "Publications relating to soils and fertilizers (duplicated), consisting of expended titles of all papers noted at the Bureau including not only the papers received at Rothamsted, but also those received at other scientific, agricultural and forest libraries to which the Bureau has access. This publication is issued monthly, price 10s. per annum.

REVIEW

Official and Tentative Methods of Analysis. Published by the Association of Official Agricultural Chemists (Washington) Third edition, 1930.

The Association of Official Agricultural Chemists, U. S. A., has just published the 3rd edition of "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists". The first edition was issued in 1920, the second in 1925 and the third is dated December 31st, 1930. The features of the new edition are the inclusion of new methods—both tentative and official—and a re-grouping of the subject matter in two divisions, *viz.*, non-foods and foods. New chapters on caustic poisons, naval stores, paints, radio-activity and egg products indicate the ever-growing scope of the Association's work.

Whatever views may be held as to the exact value of 'official' methods of analysis, a point on which there is a sharp division of opinion amongst chemists, or of the value and universal applicability of some of the methods described, no one will deny that standard methods are a necessity in connection with the day-to-day administration of Fertilizer Acts and Foods and Drugs Act. Hence this book is of the greatest value to every analyst and to every agricultural chemist; for every method described has undergone careful testing on a prescribed system before its inclusion either as an "official" or "tentative" method.

As in previous editions, methods of analysis of foodstuffs and drugs occupy by far the greater portion of the volume and this section is much more thoroughly dealt with than the chapters dealing with soils and fertilizers. Many readers will find strange the appearance in the contents page of skeleton-numbered chapter headings relating to "Sewage, Agricultural Dust, Fibres, Paper and paper material, Fish and other Marine products, Nuts and nut-products, Vitamins, Bacteriological methods and Micro-chemical methods" without there being any reading matter on these subjects. A foot-note explains that these headings indicate new activities which the Association has begun or is planning to undertake. The chapter on the analysis of soils is extremely sketchy, and agricultural chemists will not find the book of much use in this respect. Fortunately the work of the International Society of Soil Science bids fair to place soil analysis on a sound basis. The chapter on fertilizers is better but far less thorough than would have been expected. [B.C.B.]



ORIGINAL ARTICLES

STUDIES IN INDIAN TOBACCOS.

NO. 7. THE TYPES OF *NICOTIANA TABACUM* L.

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(Received for publication on the 8th June 1932.)

(With Plates XXX-XXXV)

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I.—INTRODUCTION.

India is the second largest tobacco growing country in the world. In 1927 the estimated total production of the crop in India was 1000 million pounds of which 970 million pounds was used up in the country itself [Imperial Economic Committee Report, 1928]. With such a large home market and a surplus for export it is evident that tobacco in India is an important and paying agricultural crop.

The relative importance of the provinces in the production of this crop is shown in the following table (Estimates of Area and Yield of Principal Crops in India 1929-30).

Province	Area in 1,000 acres										10 years average in 1,000 acres
	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	
Madras	201	203	214	220	265	244	232	276	255	257	236·70
Bombay (*including* Sind)	115	120	102	105	122	122	109	124	153	161	123·30
Bengal	258	298	299	288	280	293	295	290	291	295	288·70
United Provinces	65	89	89	72	73	79	75	72	81	101	79·60
Punjab	40	90	56	62	54	71	62	73	64	59	63·10
Burma	101	86	111	119	119	86	101	118	114	117	109·20
Bihar and Orissa	117	118	119	117	113	132	137	147	146	142	128·80
Central Provinces and Berar	16	24	24	20	18	17	17	19	18	14	18·70
Assam	10	11	9	9	9	9	9	10	10	10	9·60
N.-W. F. Province	..	..	..	..	..	..	..	..	11	10	10·50
Delhi	1	1	1	1	1	1	1	1	1	1	1·00
Hyderabad	135	201	201	159	140	155	116	105	99	81	139·20
Mysore	23	22	24	26	27	31	26	18	26	22	24·50
Baroda	28	31	34	28	30	27	27	22	40	45	31·20

From the above table it appears that tobacco is grown more or less all over the country but the provinces of Madras, Bombay, Bengal, Burma, Bihar and Orissa alone give seventy-five per cent. of the total production of the crop.

In Madras, Guntur is the greatest tobacco producing district and the soil here being highly retentive of moisture, tobacco is grown purely as a dry crop. In Bombay it is extensively grown in the districts of Kaira and Belgaum. At Kaira it is grown on light sandy or moderately clay loams, both with and without irrigation. At Belgaum it is grown entirely as a dry crop on black or reddish black land along the river Kistna. In Bengal the largest area centres round Rangpur on rich sandy loam along the banks of Tista river. The crop is grown on irrigation from temporary wells sunk in the field. In Burma tobacco is cultivated on the riverine areas, *i.e.*, on land which during certain months of the year is under flood water, from the Irrawady or other rivers. This consists mainly of islands in the river or a narrow strip of land on river banks. In Bihar a very

large area is cultivated in Muzaffarpur and Darbhanga districts on light sandy loams. As the soil is very retentive of moisture it is grown as a dry crop.

It appears that tobacco was introduced into India about the year 1605 [Watt] and the earliest experiments recorded with this crop date as far back as 1786 [O'Connor, 1873]. The so-called indigenous varieties of tobacco appear to have originated through crossing and inter-crossing among the types that have been introduced into India from time to time and by the selective influence of widely varying soil and climatic conditions on heterozygous types and hybrids.

In India two species of tobacco, *viz.*, *Nicotiana rustica* L. [Howard and Howard, 1910,1], yellow flowered, and *Nicotiana tabacum* L. [Howard and Howard, 1910, 2], pink flowered, are generally grown. In the Government official returns no distinction is made between the two species, it is, therefore, difficult to know accurately the individual production of each species. Of these two species *Nicotiana rustica* L. is widely cultivated in Bengal and up-country almost entirely for smoking in the country pipe (*Hooka*) and *Nicotiana tabacum* L. is grown practically all over India and supplies the major portion of the tobacco of commerce.

A previous publication [Howard and Howard, 1910, 2] deals with fifty-one different types of *Nicotiana tabacum* L., which were isolated from a large collection of Indian types growing on the Pusa Farm in 1907. The inheritance of characters and the cultivation and curing of the crop have also been dealt with in subsequent papers which are listed in the bibliography attached to this article. The present paper describes another eighteen types numbered fifty-two to sixty-nine (Plates XXX to XXXV) which have been isolated recently from a collection of mixed samples of seeds, obtained in 1925 from the leading tobacco growing centres of the country.

Localities from which the original samples of seeds were obtained and the types isolated from them are as follows :—

Province	Locality	Types isolated at Pusa
Madras	Coimbatore	60, 61
Bombay	Broach	53, 64
Do.	Kaira	55, 62, 65
Do.	Belgaum	56, 66
Bengal	Rangpur	59, 68, 69
Burma	Henzada	67
Bihar and Orissa	Darbhanga	57, 58, 63
Central India	Bhilsa	52, 54

II.—Classification and description of the types.

1. *Morphological characters.*

The chief morphological characters in which the isolated types differ are plant habit, shape of the leaves, inflorescence and flower.

Habit.—The differences in plant habit are generally caused by the length and number of internodes by the arrangement of inflorescence and by the leaves and their angle of insertion on the stem. The plants also differ in their heights and time of maturity. In classification of the types, internodes up to 2 cm. in length are classed as short, those above 2 cm. and up to 5 cm. as medium and those over 5 cm. as long.

Leaves.—The different forms of the typical leaves of the isolated types are as given below :—

Type Nos.	Forms of leaves
52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63	Sessile, elliptical.
67	Sessile, ovate.
64, 65, 66	Sessile, lanceolate.
68	Petiolate, lanceolate.
69	Petiolate, ovate.

The leaf angles in types 53, 57, 58, 59, 60, 62, 65 and 68 are rather acute, whereas in all other types they are fairly obtuse which causes the leaves to stand out horizontally from the stem.

Inflorescence.—In the majority of cases the inflorescence is open and well raised, whereas in types 52, 53, 54, 55, 62 and 67 it is rather inconspicuous and not much raised above the leaves.

Flower.—The flower colour, excepting those of types 52 and 56, varies in shades of pink. The flower colour of the former is creamy white with a slight pinkish tinge and that of the latter is almost completely white.

Calyx.—The relative lengths of the calyx and corolla do not vary much, practically in all the types the calyx is 1/3 the length of the corolla.

Corolla.—In the majority of cases the orifice varies from 6 to 10 mm., only in type 67 it is 12 mm. in diameter. In types 56, 59, 60, 61, 64 and 66 the transition between the tube and the dilated portion is gradual whereas in all other types it is abrupt. The limbs in types 52, 54, 64, 66, 67 and 69 are slightly divided but in all other types they are deeply divided.

Capsule.—In types 65 and 66 the capsules are cylindrical ; in all other cases they are conical.

Considering all the various characters in which the types differ, the leaf shape is considered the chief criterion for the main divisions in the classification with the plant habit next in importance.

2.— *Key to the classification of the types.*

I.—*Leaves sessile* :—
- (*a*) Elliptical—
  - Internodes short—
    - Inflorescence rather inconspicuous—
      - Plants very early, dwarf . . . . . Type 52
      - Plants very early, medium in height . . . . . Type 53
      - Plants early, rather medium in height . . . . Type 54
      - Plants late, dwarf . . . . . Type 55
    - Inflorescence rather compact and slightly raised—
      - Plants very early, dwarf . . . . . Type 56
    - Inflorescence open and much raised—
      - Plants early, tall . . . . . Type 57
  - Internodes medium—
    - Inflorescence open and well raised—
      - Plants early, tall . . . . . Type 58
      - Plants rather late, tall . . . . . Type 59
      - Plants late, medium in height . . . . . Type 60
      - Plants very late, tall . . . . . Type 61
  - Internodes long—
    - Inflorescence rather compact and not much raised—
      - Plants early, tall . . . . . Type 62
    - Inflorescence open and well raised—
      - Plants early, tall . . . . . Type 63
- (*b*) Lanceolate—
  - Internodes medium—
    - Inflorescence open and well raised—
      - Plants very early, medium in height . . . . Type 64
      - Plants early, medium in height . . . . . Type 65
      - Plants early, tall . . . . . Type 66
- (*c*) Ovate—
  - Internodes long—
    - Inflorescence rather compact and not much raised—
      - Plants late, tall . . . . . Type 67

II. *Leaves petiolate* :—
- (*a*) Lanceolate—
  - Internodes short—
    - Inflorescence open and well raised—
      - Plants early, tall . . . . . Type 68
- (*b*) Ovate—
  - Internodes long—
    - Inflorescence open and well raised—
      - Plants early, tall . . . . . Type 69

3. *Description of the types.*

Type 52. —Plants very early, much dwarf, average height 60 cms., internodes short, leaves borne at an equal distance on the stem. *Leaves* sessile, inserted at an

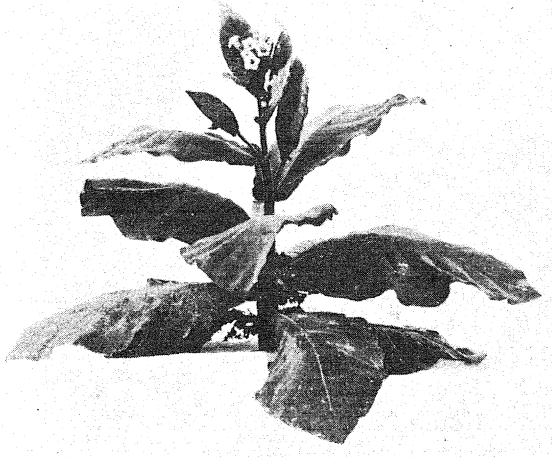
angle of 90°, amplexicaul, auriculate, decurrent, decurrency broad; elliptical, lamina narrowed very near the base; secondary veins arise at an angle of 60°; apex acute; margin wavy; surface flat; colour dark green, average length 60 cms., ratio length/breadth 2·2. *Inflorescence leaves* elliptical but smaller in size. *Inflorescence* inconspicuous and not much raised above the leaves, side branches grow as tall as the main axis. *Flowers* almost white with pinkish tinge, big, thickly arranged on short stalks. *Calyx* globular and much inflated less than $\frac{1}{3}$ the length of the corolla; teeth short and pointed. *Corolla* with an orifice of 10 mm. in diameter; tube very broad; transition between the tube and dilated portion abrupt; limb slightly divided with slight folds at the junction of the lobes; lobes rounded at the base; apical points short. *Capsule* conical, nearly $\frac{2}{3}$ covered with the persistent calyx; apex somewhat pointed.

Anthers burst before the flowers open and are in level with the stigma. In the fully opened flowers the anthers remain below the orifice.

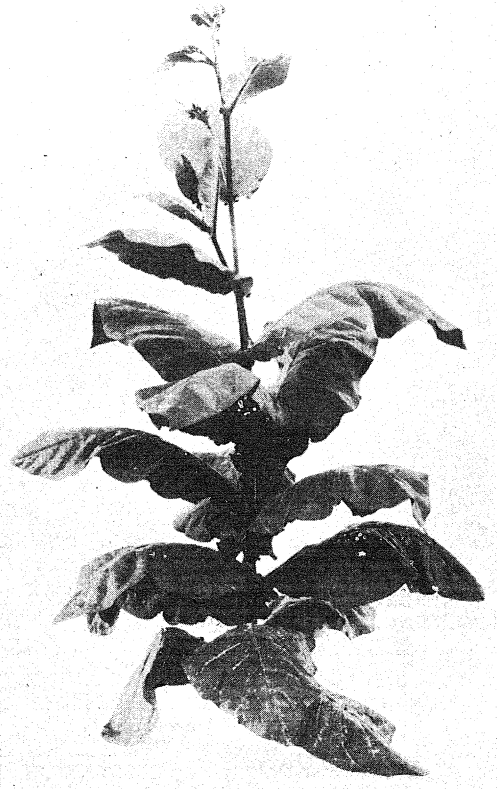
Type 53.—Plants very early, medium in height; average height 120 cms., internodes short, leaves closely borne on the stem. *Leaves* sessile, inserted at an angle of 60°; amplexicaul and decurrent, decurrency broad; elliptical, lamina narrowed very near the base and drooping at about $\frac{1}{3}$ from the apex; secondary veins arise at an angle of 60°, apex acute; margin wavy; surface slightly raised between secondary veins; colour somewhat dark green; texture medium; average length 39 cm.; ratio length/breadth 1·8. *Inflorescence leaves* elliptical but smaller in size. *Inflorescence* rather inconspicuous and not much raised, secondary branches grow as tall as the main axis. *Flowers* light pink and thickly arranged on short stalks. *Calyx* globular and much inflated, a little over $\frac{1}{3}$ the length of the corolla; teeth long and pointed. *Corolla* with an orifice of 7 mm. in diameter; tube medium; transition between the tube and dilated portion abrupt; limb deeply divided with folds at the junction of the lobes; lobes somewhat rounded at the base; apical points short and pointed. *Capsule* conical, almost covered with persistent calyx, teeth of calyx projecting beyond the capsule; apex blunt.

Anthers burst before the flower opens and they are in level with the stigma. Both anthers and stigma reach the orifice.

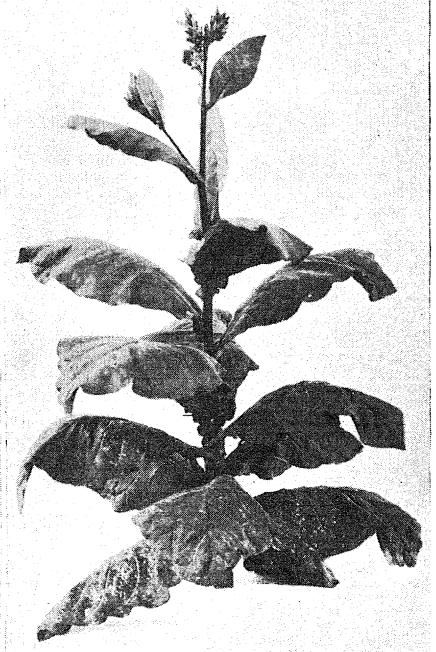
Type 54.—Plants early, rather medium; average height 100 cms., internodes short, leaves borne at an equal distance on the stem. *Leaves* sessile, inserted at an angle of 90°, amplexicaul, decurrent, decurrency broad; elliptical, lamina narrowed at about 10 cms., from the base; secondary veins arise at an angle of 60°; apex acute; margin wavy; surface somewhat raised between secondary veins; colour dark green; texture fairly thick; average length 45 cms., ratio length/breadth 1·6. *Inflorescence leaves* elliptical but smaller in size. *Inflorescence* rather inconspicuous and not much raised above the leaves, side branches grow as tall as the main axis.



Type 52.



Type 53.



Type 54.

PLATE XXX



Type 55. Type 56. Type 57.

Flowers medium, colour light pink, thickly arranged on short stalks. *Calyx* globular and much inflated, a little less than $\frac{1}{3}$ the length of the corolla ; teeth short and pointed. *Corolla* with an orifice of 8 mm. in diameter, tube medium ; transition between the tube and the dilated portion abrupt ; limb slightly divided and recurved with slight folds at the junction of the lobes ; lobes rounded at the base ; apex short and oblique. *Capsule* conical, nearly $\frac{3}{4}$ covered with persistent calyx ; apex pointed.

Anthers burst before the flower opens and remain below the stigma. In the fully opened flowers the stigma is just projecting beyond the orifice.

Type 55.—Plants rather late, dwarf ; average height 90 cms., internodes short, leaves borne very close on the stem. *Leaves* sessile, inserted at an angle of 90°, amplexicaul and slightly decurrent ; elliptical, lamina narrowed at about 10 cms. from the base ; secondary veins arise at an angle of 60 ; apex acute ; margin slightly undulate ; surface somewhat raised ; colour dark green ; texture thick ; average length 60 cms., ratio length/breadth 1·2. *Inflorescence leaves* elliptical. *Inflorescence* rather inconspicuous and not much raised, side branches grow as tall as the main axis. *Flowers* light pink, colour rather conspicuous at the apical points, thickly arranged on short stalks. *Calyx* globular and inflated less than $\frac{1}{3}$ the length of the corolla ; teeth short and acute. *Corolla* with an orifice of 8 mm. in diameter ; tube abroad ; transition between the tube and dilated portion abrupt ; limb divided at about half its depth with slight folds at the junction of the lobes ; lobes rounded at the base ; apical points short. *Capsule* conical, nearly $\frac{2}{3}$ of it covered with persistent calyx ; apex pointed.

Anthers burst before the flower opens and remain well above the stigma. In fully opened flower, both the anthers and stigma are in level with orifice.

Type 56.—Plants very early, dwarf ; average height 95 cms., internodes short, practically all the leaves borne very near the base. *Leaves* sessile, inserted at an angle of 90°, amplexicaul and slightly decurrent, decurrency very narrow ; elliptical, lamina narrowed at about 12 cms. from the base ; secondary veins arise at an angle of 30° and are fairly close ; apex accuminate ; margin practically entire ; surface smooth ; colour light green ; texture medium ; average length 45 cms., ratio length/breadth 2·1. *Inflorescence leaves* lanceolate. *Inflorescence* rather compact and slightly raised above the leaves, side branches grow as tall as the main axis. *Flowers* medium, creamy white with slight pinkish tinge at the apical points and the margin of the lobes and thickly arranged at the top of short stalks. *Calyx* tubular, slightly less than half the length of the corolla ; teeth rather long and acute. *Corolla* with an orifice of 8 mm. in diameter ; tube medium ; transition between the tube and the dilated portion gradual ; limb divided to about $\frac{2}{3}$ of its depth, with slight folds at the junction of the lobes ; lobes triangular shaped at the

base; apical points long. *Capsule* conical; almost covered with persistent calyx; teeth projecting beyond the capsule; apex pointed.

Anthers burst before the flower opens and are in level with stigma. In the fully opened flowers the stigma slightly projects above the orifice.

In the early flowers the anthers have very scanty pollen and hence setting is very little, but later when the season gets warm, anthers produce much pollen and result in good setting of seeds.

Type 57.—Plants early, tall; average height 170 cms., lower internodes short, most of the leaves borne very near the base in a rosette form. *Leaves* sessile, inserted at an angle of 60°, amplexicaul, decurrent, decurrency very narrow; elliptical, lamina narrowed at about 15 cms. from the base and folding on midrib; secondary veins arise at an angle of 45°; apex accuminate; margin undulate and recurved especially towards the base; surface rough; colour dark green; texture thick; average length 170 cms., ratio length/breadth 2·9. *Inflorescence leaves* lanceolate. *Inflorescence* well open and much raised on long slender branches above the leaves; side branches grow as tall as the main axis. *Flowers* pink and sparse, borne on long slender branches. *Calyx* tubular somewhat inflated and less than half the length of the corolla; teeth long and pointed. *Corolla* with an orifice of 8 mm. in diameter, tube medium; transition between the tube and the dilated portion abrupt; limb divided with slight folds at the junction of the lobes; lobes rounded at the base; apical points medium and pointed. *Capsule* conical about $\frac{2}{3}$ covered with persistent calyx; apex blunt.

Anthers burst before the flowers open and are well above the stigma. Only the anthers reach the orifice and the stigma remains much below the orifice.

Type 58.—Plants early, tall; average height 162 cms.; internodes medium, most of the leaves borne on the lower half of the stem. *Leaves* sessile, inserted at an angle of 60° amplexicaul, slightly auriculate, decurrent, decurrency not very broad; elliptical, lamina narrowed at about 12 cms. from the base and slightly folded on midrib; secondary veins arise at an angle of 45°; apex acute; margin undulate; surface much puckered; colour dark green; texture very thick; average length 50 cms., ratio length/breadth 2·2. *Inflorescence leaves* elliptical but smaller in size. *Inflorescence* open and well raised on long slender branches above the leaves, secondary branches remain below the main axis. *Flowers* pink and sparsely arranged on short stalks. *Calyx* somewhat tubular about $\frac{1}{3}$ the length of the corolla; teeth long and pointed. *Corolla* with an orifice of 10 mm. in diameter; tube medium; transition between the tube and the dilated portion abrupt; limb deeply divided with slight folds at the junction of the lobes; lobes slightly rounded at the base: apical points short and pointed. *Capsule* conical about $\frac{2}{3}$ covered with persistent calyx; apex blunt.



Type 58.

Type 59.

Type 60.

PLATE XXXII

Anthers burst before the flower opens and are well above the stigma. In the fully opened flowers the anthers and stigma remain much below the orifice.

Type 59.—Plants rather late, tall ; average height 190 cms.,internodes medium, leaves borne at an equal distance on the stem. *Leaves* sessile, inserted at an angle of 60°, amplexicaul and auriculate, slightly decurrent ; elliptical, lamina much narrowed at about 10 cms. from the base, drooping at about the middle and slightly folded on midrib ; secondary veins arise at an angle of 30° ; apex acuminate ; margin undulate and recurved ; surface somewhat smooth ; colour light green ; texture thick ; average length 60 cms., ratio length/breadth 2·2. *Inflorescence leaves* lanceolate. *Inflorescence* open and much raised on long slender branches and the side branches grow as tall as main axis. *Flowers* pink, and clustered at the ends of short stalks. *Calyx* cylindrical, inflated, less than $\frac{1}{3}$ the length of the corolla ; teeth long and accuminate. *Corolla* with an orifice of 8 mm. in diameter ; tube broad, the transition between the tube and the dilated portion gradual ; limb divided deep down toward the base, with folds at the junction of the lobes ; lobes triangular shaped at the base ; apical points very long. *Capsule* conical, nearly half covered with persistent calyx ; apex pointed.

Anthers burst before the flower opens and remain well above the stigma. In the fully opened flowers both the anthers and stigma are just in level with the orifice.

Type 60.—Plants late, medium in height ; average height 115 cms. ; lower internodes short, upper medium, leaves borne practically at an equal distance on the stem. *Leaves* sessile, inserted at an angle of 60°, amplexicaul and decurrent, decurrency not very broad ; elliptical lamina narrowed at about 12 cm. from the base ; secondary veins arise at an angle of 60° ; apex acute ; margin slightly undulate ; surface flat ; colour dark green ; texture medium ; average length 50 cms., ratio length/breadth 2·0. *Inflorescence leaves* lanceolate. *Inflorescence* open, well raised above the leaves, the secondary branches grow as tall as the main axis. *Flowers* light pink and thickly arranged on the top of short stalks. *Calyx* tubular, slightly inflated, about $\frac{1}{3}$ the length of the corolla ; teeth long and pointed. *Corolla* with an orifice of about 8 mm. in diameter ; tube medium ; transition between the tube and dilated portion gradual ; limb deeply divided with folds at the junction of the lobes and recurved; lobes slightly rounded at the base ; apical points long and twisted. *Capsule* conical, about $\frac{2}{3}$ covered with persistent calyx, teeth of calyx projecting beyond the capsule ; apex pointed.

Anthers burst before the flower opens and they are practically in level with the stigma. In the fully opened flowers the anthers and stigma remain a little below the orifice.

Type 61.—Plants very late, tall; average height 160 cms., internodes medium, leaves borne at an equal distance on the stem. *Leaves* sessile, inserted at an angle

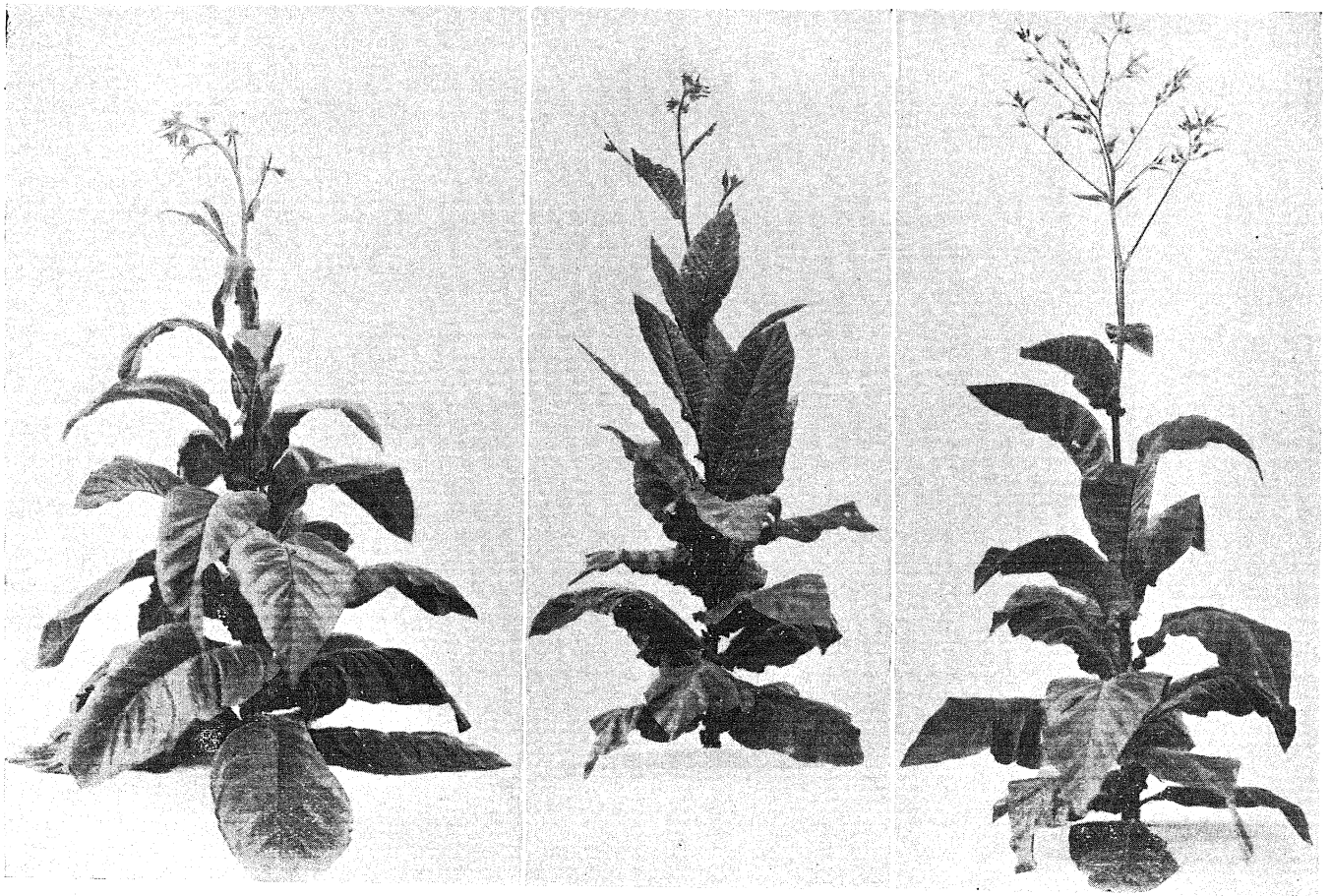
of 75°, amplexicaul, decurrent, decurrency somewhat broad ; elliptical, lamina narrowed at about 12 cms., from the base and not fully expanded ; secondary veins arise at an angle of 60° ; apex acute ; margin undulate ; surface slightly folded between the secondary veins; colour dark green ; texture somewhat thick ; average length 45 cms., ratio length/breadth 2·1. *Inflorescence leaves* lanceolate. *Inflorescence* open and much raised, the side branches grow as tall as the main axis. *Flowers* pink and sparsely arranged on short stalks. *Calyx* tubular and inflated nearly $\frac{1}{3}$ the length of the corolla ; teeth long and accuminate. *Corolla* with an orifice of 8 mm. in diameter ; tube broad ; transition between the tube and the dilated portion gradual ; limb deeply divided with folds at junction of lobes ; lobes rounded at the base ; apical points long and oblique. *Capsule* conical, about $\frac{2}{3}$ of it covered with persistent calyx ; apex pointed.

Anthers burst before the flower opens and remain in level with the stigma. In the fully opened flowers both the anthers and stigma remain below the orifice.

Type 62.—Plants early, tall ; average height 170 cms., internodes moderately long, leaves borne at an equal distance on the stem. *Leaves* sessile, inserted at an angle of 60°, amplexicaul and decurrent, decurrency not very broad ; elliptical ; lamina narrowed very near the base ; secondary veins arise at an angle of 60° ; apex acute ; margin slightly undulate ; surface somewhat puckered ; colour dark green ; texture thick ; average length 44 cms., ratio length/breadth 1·6. *Inflorescence leaves* elliptical but smaller in size. *Inflorescence* rather compact and not much raised above the leaves, side branches grow as tall as the main axis. *Flowers* pink, many and thickly arranged in bunches on short stalks. *Calyx* globular and inflated, a little less than half the length of the corolla ; teeth short and acute. *Corolla* with an orifice of 7 mm. in diameter, tube bent ; transition between the tube and dilated portion abrupt ; limb deeply divided with folds at the junction of the lobes ; lobes rounded at the base ; apical points rather short. *Capsule* conical, nearly $\frac{2}{3}$ covered with persistent calyx ; apex pointed.

Anthers burst before the flower opens and they are practically in level with the stigma. In the fully opened flowers the anthers and stigma reach the orifice.

Type 63.—Plants early, tall ; average height 166 cms., lower internodes very short, upper long ; most of the leaves borne very near the base. *Leaves* sessile, inserted at the angle of 90°, amplexicaul, and decurrent, decurrency not very broad ; elliptical, lamina narrowed at about 12 cms. from the base ; secondary veins arise at an angle of 60° ; apex acute ; margin undulate ; surface slightly raised between the veins ; colour dark green, texture thick ; average length 55 cms., ratio length/breadth 2·0. *Inflorescence leaves* elliptical. *Inflorescence* open, and well raised above the leaves, secondary branches grow as tall as the main axis. *Flowers* pink, sparse and arranged on the top of short stalks. *Calyx* tubular and slightly



Type 61.

Type 62.

Type 63.

PLATE XXXIII

PLATE XXXIV



Type 66.

Type 65.

Type 64.

inflated, less than half the length of the corolla; teeth long and pointed. *Corolla* with an orifice of 10 mm. in diameter; tube medium and slightly bent; transition between the tube and dilated portion abrupt; limb deeply divided with folds at the junction of the lobes; lobes rounded at the base; apical points rather long and pointed; *capsule* conical, nearly $\frac{3}{4}$ covered with persistent calyx; apex blunt.

Anthers burst before the flower fully opens and they are in level with stigma. In the fully opened flowers the anthers and stigma remain a little below the orifice.

Type 64.—Plants very early, medium in height; average height 130 cms., internodes medium, leaves borne at an equal distance on the stem. *Leaves* sessile, inserted at an angle of 90°, amplexicaul, decurrent, lanceolate; lamina narrowed very near the base; secondary veins arise at an angle of 45°; apex acute; margin slightly undulate; surface somewhat puckered; colour light green, texture thin; average length 40 cms., ratio length/breadth 2·5. *Inflorescence leaves* lanceolate. *Inflorescence* much open and well raised on the long slender branches, side branches grow as tall as the main axis. *Flowers* medium, rather bent, pink, arranged closely and form a bunch on the top of short stalks. *Calyx* tubular, much inflated, a little less than half the length of the corolla; teeth short and pointed. *Corolla* with an orifice of 7 mm. in diameter; tube slender and bent; transition between the tube and dilated portion gradual; limb slightly divided with nominal folds at the junction of the lobes; lobes triangular shaped at the base; apical points short. *Capsule* conical, the teeth of the persistent calyx projecting beyond the capsule; apex pointed and compressed.

Anthers burst before the flower opens and are in level with the stigma but they never reach the orifice.

Type 65.—Plants early, medium in height, average height 130 cms., internodes medium, leaves borne at an equal distance on the stem. *Leaves* sessile, inserted at an angle of 60°, amplexicaul, slightly decurrent, auriculate; lanceolate, lamina narrowed at about 12 cms. from the base; secondary veins arise at an angle of 45°; apex accuminate; margin slightly wavy; surface slightly folded on midrib; colour light green; texture thick; average length 55 cms., ratio length/breadth 2·6. *Inflorescence leaves* lanceolate. *Inflorescence* open and well raised above the leaves, side branches grow as tall as the main axis. *Flowers* rather bent, pink, colour more prominent at apical points, thickly arranged on short stalks. *Calyx* tubular and inflated less than half the length of the corolla; teeth rather long. *Corolla* with an orifice of 8 mm. in diameter; tube medium; transition between the tube and dilated portion somewhat abrupt; limb divided to about half its depth, with folds at the junction on the lobes; lobes rounded at the base; apical points short. *Capsule* cylindrical, nearly $\frac{2}{3}$ covered with persistent calyx; apex pointed.

In some cases the anthers are in level with stigma but in majority they are

much below the stigma. In fully opened flowers the stigmas project beyond the orifice.

Type 66.--Plants early, tall, average height 160 cms., internodes medium, leaves borne at an equal distance on the stem. *Leaves* sessile, inserted at an angle of 90°, amplexicaul, decurrent, decurrency broad; lanceolate, lamina narrowed at about 10 cms. from the base; secondary veins arise at an angle of 60°, apex accuminate; margin wavy; surface nearly flat; colour light green; texture fairly thick; average length 60 cms., ratio length/breadth 2·4. *Inflorescence leaves* lanceolate. *Inflorescence* open and much raised above the leaves, side branches grow as tall as the main axis. *Flowers* medium, rather bent, pink and arranged in bunches on short stalks. *Calyx* tubular and inflated, a little less than half the length of the corolla; teeth long and pointed. *Corolla* with an orifice of 6 mm. in diameter; tube slender; transition between the tube and dilated portion almost gradual; limb slightly divided; lobes triangular shaped at the base; apical points long. *Capsule* cylindrical, nearly the whole of it covered with persistent calyx, the calyx projecting beyond the capsule; apex pointed.

Anthers are in level with the stigma. In fully opened flowers the anthers and stigma never reach the orifice.

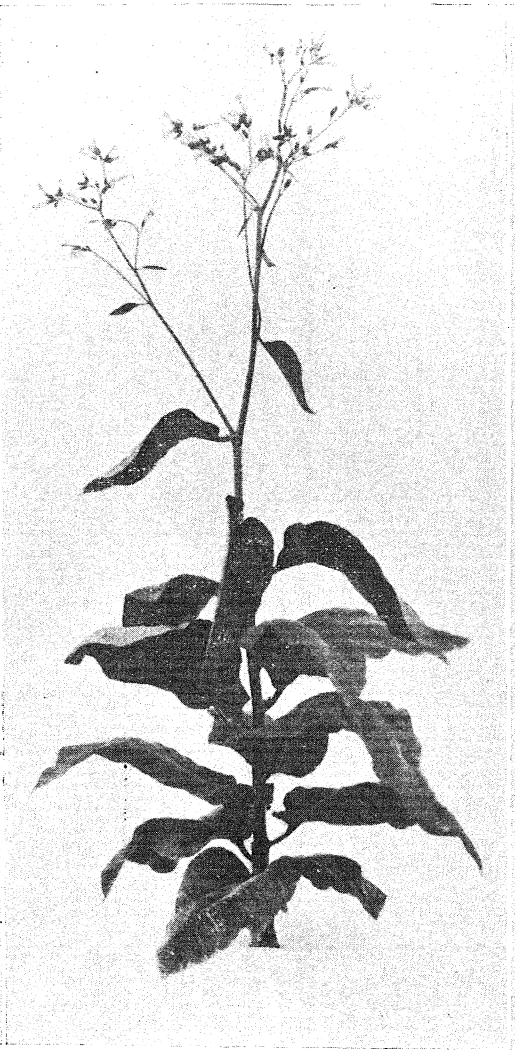
Type 67.—Plants late, tall; average height 160 cm., internodes long, leaves borne at an equal distance on the stem. *Leaves* sessile inserted at an angle of 90°, amplexicaul, decurrent, decurrency very broad; ovate, lamina narrowed very near the base; secondary veins arise at an angle of 60°; apex acute; margin undulate; surface slightly raised between the secondary veins; colour dark green; texture very thick; average length 50 cm., ratio length/breadth 1·7. *Inflorescence leaves* ovate to elliptical but smaller in size. *Inflorescence* rather compact and not much raised above the leaves, secondary branches remain below the main axis. *Flowers* light pink and arranged in bunches on short stalks. *Calyx* globular and much inflated, little less than half the length of the corolla; teeth short and pointed. *Corolla* with an orifice of about 12 mm. in diameter; tube very broad; transition between the tube and dilated portion abrupt; limb slightly divided; apicule short and pointed. *Capsule* conical, nearly $\frac{2}{3}$ covered with persistent calyx; apex pointed.

Anthers burst before the flower opens and remain in level with the stigma. In fully opened flowers the anthers and stigma project beyond the orifice.

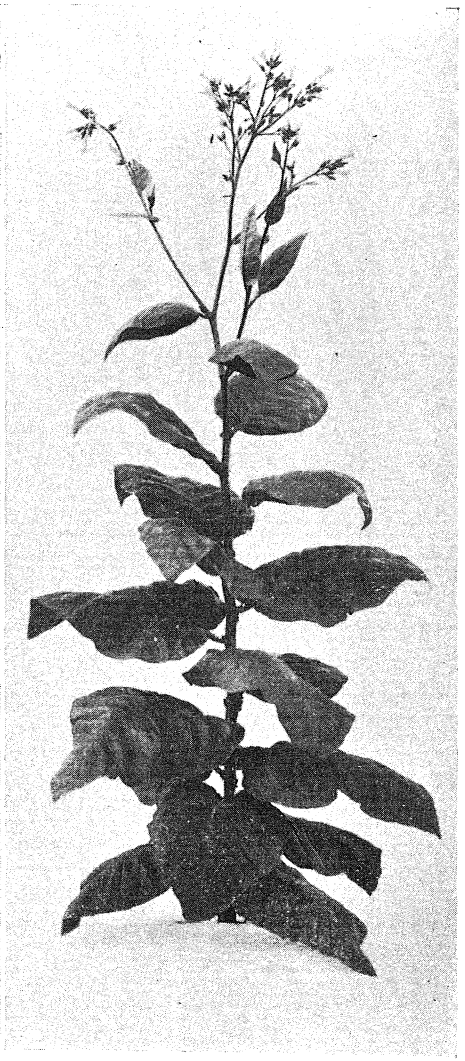
Type 68.—Plants early, tall; average height 170 cm., lower internodes short, upper long, most of the leaves borne very near the base. *Leaves* petiolate, inserted at an angle of 60°; lanceolate, secondary veins arise at an angle of 60°; apex acute and invariably notched; margin wavy; surface slightly raised between the secondary veins; colour dark green; texture thick; average length 50 cm., ratio length/breadth 2·0. *Inflorescence leaves* lanceolate. *Inflorescence* much open and



Type 67.

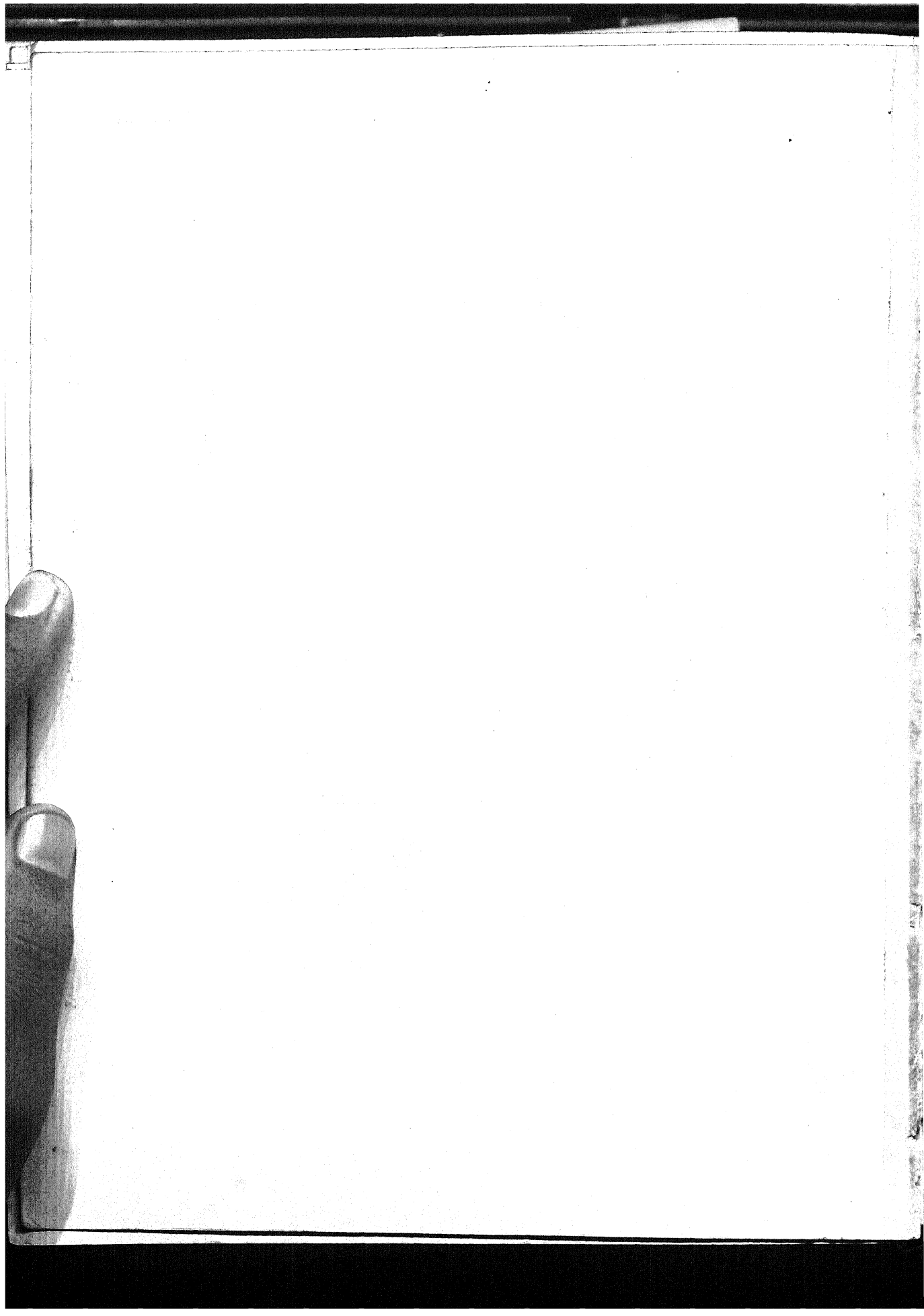


Type 68.



Type 69.

PLATE XXXV



well raised on long slender branches above the leaves, side branches remain below the main axis. *Flowers* pale pink, sparse, arranged far apart on the top of the short stalks. *Calyx* globular and inflated, slightly less than half the length of the corolla; teeth short and acute. *Corolla* with an orifice of 8 mm. in diameter; tube medium; transition between the tube and the dilated portion abrupt; limb deeply divided and recurved with slight folds at the junction of the lobes; lobes somewhat triangular shaped at the base; apical points long. *Capsule* conical, about $\frac{2}{3}$ covered with persistent calyx; apex pointed.

Anthers burst before the flower opens and remain in level with the stigma. In fully opened flowers the anthers and stigma just reach the orifice.

Type 69.—Plants early, tall; average height 160 cm., internodes long, leaves borne at an equal distance on the stem. *Leaves* petiolate with slight alate petioles, inserted at an angle of 90°; ovate; secondary veins arise at an angle of 60°; apex acute; margin slightly undulate; surface somewhat raised between the veins; colour light green; texture thin; average length 45 cm., ratio length/breadth 1·4. *Inflorescence leaves* ovate but smaller in size. *Inflorescence* open and well raised above the leaves, side branches grow as tall as the main axis. *Flowers* small, pink, arranged fairly apart on the top of short stalks. *Calyx* globular and inflated, less than $\frac{1}{3}$ the length of the corolla; teeth short. *Corolla* with an orifice of 7 mm. in diameter; tube medium; transition between the tube and dilated portion abrupt; limb somewhat divided with slight folds at the junction of the lobes; lobes rounded at the base; apical points short. *Capsule* conical, nearly $\frac{3}{4}$ covered with persistent calyx; apex pointed.

Anthers burst before the flower opens and are well above the stigma. In fully opened flowers the anthers and stigma are in level with the orifice.

REFERENCES.

1. Estimates of Area and Yield of Principal Crops in India 1929-30.
2. Howard, A. and Howard, G. L. C. (1910, 1). *Mem. Dept. Agric. Ind., Bot. Ser.* **3.**
3. ——— ——— ——— (1910, 2). *Mem. Dept. Agric. Ind., Bot. Ser.* **3.**
4. ——— ——— ——— ——— (1913). *Mem. Dept. Agric. Ind., Bot. Ser.* **4,** No. 3.
5. ——— ——— ——— ——— (1915). *Agric. Res. Inst, Pusa, Bull. 50.*
6. Howard, G. L. C. and Kashi Ram (1924). *Mem. Dept. Agric. Ind., Bot. Ser.* **13,** No. 1.
7. Imperial Economic Committee Report (1928).
8. O'Connor, J. E. (1873). Report on the Production of Tobacco in India.
9. Kashi Ram (1931). *Ind. J. Agric. Sci.* **1,** 44—5472.
10. Shaw, F. J. F. (1928). Capital Industrial Supplement.
11. Shaw, F. J. F., and Kashi Ram (1928). *Agric. Res. Inst., Pusa, Bull.* 187.
12. Watt, Sir George. The Commercial Products of India.

C

OBSERVATIONS ON THE IMMATURE STAGES OF SOME INDIAN PSYLLIDÆ

[HOMOPTERA: RHYNCHOTA]

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(With Plates XXXVI—XL).

INTRODUCTION.

Buckton [1893, 1900], Lefroy [1909], and more extensively Crawford [1912, 1924], have described and figured a number of the adults of the Indian Psyllidæ but the nymphal forms have so far received comparatively little attention. Grove and Ghosh [1914] gave a brief description of the nymphal forms of *Arytaina punctipennis* Craw. (Sub-family: PSYLLINÆ) and Afzal Husain and Dina Nath [1929] described very fully the immature forms of *Diaphorina citri* Kuw. (Sub-family: PSYLLINÆ.)

The importance of a study of the nymphal forms, both from the point of control and taxonomy, cannot be over-emphasized. Considerable stress has been laid on the taxonomic value of such a study by Crawford [1914] and Ferris [1923]. With our present knowledge of the nymphs of the Indian Psyllidæ it is very difficult, if not impossible, to correctly identify even some of the common pests in their immature stages.

Crawford's [1914] classification of the family Psyllidæ based entirely on the characters of the adult—the shape of head and genæ, and wing venation—* does not appear to be entirely satisfactory. He divides Psyllidæ into the following six sub-families :—

1. LIVIINÆ
2. PAUROPSYLLINÆ
3. CARSIDARINÆ
4. CERIACREMINÆ
5. TRIOZINÆ
6. PSYLLINÆ

Regarding the sub-family TRIOZINÆ he [1919] remarks: "the most easily visible, though not the most constant, character of this sub-family is the point of furcation of the basal vein of the forewing—the cubitus, media and radius leaving the basal vein at quite or nearly the same point. *Ceropsylla* and *Hemitrioza*, both American genera, are exceptions to this. On the other hand, several species belonging to other sub-families, possess this characteristic, as follows: *Rhinopsylla* and *Tenaphalara triozipennis*, of the sub-family CARSIDARINÆ; and *Pauropsylla triozoptera* and *Leptynoptera*, of the sub-family PAUROPSYLLINÆ."

*Crawford [1914] himself is of opinion that the character of wing venation may prove to be valueless as a sub-family character (p. 62)

The study of the nymphal forms shows still more clearly the unsatisfactory nature of this classification. Ferris [1928, 1] regarding *Synoza floccosa* Ferris (Sub-family : CARSIDARINÆ) remarks : " the knowledge of the nymphs of this sub-family is still too fragmentary to permit any conclusions concerning the evidence that they may afford as to relationships. I would merely call attention to the fact that this nymph bears extremely little resemblance to that next to be described (*Freysuila cohahuayanæ* Ferris), although on the basis of existing classification the two species are referred to the same sub-family ". Afzal Husain and Dina Nath [1929] state that " the nymphal affinities of *Diaphorina citri* Kuw. suggest a closer relationship with the sub-family TRIOZINÆ rather than with the sub-family PSYLLINÆ ", to which sub-family on the basis of Crawford's classification this Psyllid belongs. The nymphal forms of *Tenaphalara elongata* Craw. (Sub-family : CARSIDARINÆ) (Plate XXXVII), show a greater affinity with nymphs of the sub-family PSYLLINÆ as wing-pads are not produced cephalad but stand out prominently from the general contour of the body.

Again, while placing the genus *Tenaphalara* Kuw. in the sub-family CARSIDARINÆ, Crawford [1919] says " this is rather an anomalous genus, suggestive of *Aphalara* and also *Pauropsylla* in the head shape, and of CARSIDARINÆ in wing venation, tarsi, and eyes ". *Aphalara* is placed in the sub-family LIVIINÆ while *Pauropsylla* is a typical genus of the sub-family PAUROPSYLLINÆ. The study of the nymphs of these two genera on the other hand clearly separate *Tenaphalara* from *Aphalara* and *Pauropsylla i.e.*, nymphs of the former are of the typical psylliine type and the two latter do not belong to this type.

Ferris [1925], as a result of his study of a number of forms, recognizes two distinct types of nymphs in this family (1) the *triozine* type, " in which the wing-pads are produced cephalad at the humeral angle and otherwise so arranged that their margin is more or less continuous with the margins of head and abdomen." (2) the *psylliine* type, " in which the wing-pads are not produced cephalad at the humeral angle and they project prominently from the contour of the body."

Ferris's [1925] grouping of the nymphs of this family, on account of the limited number of nymphal forms studied, has by no means reached finality. In the nymphs of *Pauropsylla tuberculata* Craw. humeral angle of front wing-pads are rounded but not produced cephalad as is the case with *Trioza fletcheri* Craw. (Plate XXXII), although there is a distinct tendency towards that condition. Margins of front wing-pads run in line with the general contour of the body. It, therefore, seems necessary to add another type to the two types of Ferris and call it *Pauropsylline* type, in which the front wing-pads are not produced cephalad at the humeral angle but show a tendency in that direction, their outer margins are however, in line with the general contour of the body. The immature forms of

Pauropsylla depressa Craw. (Sub-family : PAUROPSYLLINÆ), and *Leuronota michoacana* Ferris (Sub-family : TRIOZINÆ) studied by Ferris [1928, 2] and *Aphalara calthæ* L. (Sub-family : LIVIINÆ) studied by Klyver [1930], fall under this type.

So far the nymphs of the following species have been studied in detail :—

Sub-family	Name	Nymphal type	Reference
PAUROPSYLLINÆ	1. *Pauropsylla tuberculata* Craw.	Pauropsylline	Present paper
	2. *Pauropsylla depressa* Craw.	Do.	Present paper
CARSIDARINÆ	3. *Synoza floccosa* Ferris	Psylliine	Ferris 1928, 1
	4. *Freysuila cohahuayanae* Ferris	Do.	Ferris 1928, 1
	5. *Carsidara gigantea* Craw.	Do.	Ferris 1928, 2
	6. *Tenaphalara elongata* Craw.	Do.	Present paper
PSYLLINÆ	7. *Euphyllura arbuti* Sch.	Do.	Ferris and Hyatt 1923
	8. *Psyllopsis fraxinicola* Foer.	Do.	Ferris 1923
	9. *Psylla alni* Linn.	Do.	Ferris 1925
	10. *Psylla buxi* Linn.	Do.	Ferris 1926
	11. *Pachypsylla venusta* Osten Sacken	Do.	Ferris 1926
	12. *Euphalerus gallicola* Ferris	Do.	Ferris 1928, 1
	13. *Euphyllura arctostaphyli* Sch.	Do.	Ferris 1928, 1
	14. *Arytaina punctipennis* Craw.	Do.	Present paper
	15. *Diaphorina citri* Kuw.	Triozine	Afzal Hussain and Dina Nath 1929
TRIOZINÆ	16. *Ceropsylla sideroxyli* Riley	Do.	Ferris 1923
	17. *Trioza urticae* Linn.	Do.	Ferris 1925 ; Klyver 1930
	18. *Paratrioza cockerelli* Sulc.	Do.	Ferris 1925
	19. *Phyllopecta diospyri* Ashmead	Do.	Ferris 1926
	20. *Leuronota michoacana* Ferris	Pauropsylline (Triozine type, Ferris)	Ferris 1928, 2
	21. *Trioza albifrons* Craw.	Triozine	Klyver 1930
	22. *Trioza fletcheri* Craw.	Do.	Present paper
LIVIINÆ	23. *Aphalara calthae* L.	Pauropsylline (Psylliine type, Klyver)	Klyver 1930

From the above it will be seen that on the basis of the form of the nymph, family Psyllidæ is classifiable into three groups, *viz.*, 1. Pauropsylline, 2. Psylliine, 3. Triozine. Nymphs of the sub-family CARSIDARINÆ resemble the nymphs of the sub-family PSYLLINÆ; and the single nymph of the sub-family LIVIINÆ (so far studied) and the nymph of *Leuronota michoacana* Ferris of the sub-family TRIOZINÆ resemble the nymphs of the sub-family PAUROPSYLLINÆ; and the nymphs of *Diaphorina citri* Kuw. of the sub-family PSYLLINÆ resemble the nymphs of the sub-family TRIOZINÆ. With the advancement of our knowledge about the immature forms of Psyllidæ the classification of the family will have to be revised.

ACKNOWLEDGMENTS.

This line of work was suggested by Mr. M. Afzal Husain and was started under his guidance during his tenure of office as Officiating Imperial Entomologist, 1925-26, Pusa, to whom the author expresses his sincerest thanks for suggesting the problem, for help during the progress of this work and in writing up this paper. I am also thankful to Mr. T. Bainbrigge Fletcher, Imperial Entomologist, Pusa, for his guidance and suggestions and for allowing me to remove material to Lyallpur on my transfer. I must also express appreciation of Mr. Narainam, Artist, Agricultural Research Institute, Pusa, for his sketching Plate XXXVI, of Mr. M. D. Siddiqi, Artist, Entomological Section, Lyallpur, for sketching Plates XXXVII, XXXVIII, XXXIX, and of Mr. S. M. Nasir, in-charge Photo Section, P. A. College, Lyallpur, for his taking the photographic illustrations.

PAUROPSYLLA TUBERCULATA CRAW. (Sub-family : PAUROPSYLLINÆ).

(Plate XXXVI.)

Material.—Eggs and nymphs were collected between 27th March to 18th November 1926 (Colls. M. Afzal Husain and K. A. R.) from leaves of *Alstonia scholaris* R. Br., Imperial Agricultural Research Institute, Pusa (India).

The mature gall (Plate XXXVI, figs. 6, 6g, 6gt).—The nymphs form galls on the leaves of *Alstonia scholaris* R. Br. A mature gall is a small barrel-like structure and is 3·5 mm. long and 2 mm. broad across the top (Plate XXXVI, fig. 6g). A larger part of the 'barrel' projects below the lower surface of the leaf lamina. The surface of the gall is smooth and its colour harmonizes with the colour of the respective side of the leaf, *i.e.*, the round bottom which is towards the upper side of leaf is green, and the flat top portion towards the lower side is whitish-green. In the middle of the lower end is a slit (Plate XXXVI, fig. 6gt) that marks the opening of the gall, the exit for the nymph.

The galls are irregularly scattered and are usually isolated, but sometimes two or more occur together forming a group (Plate XXXVI, fig. 6) although each gall is an independent entity with its own exit, and invariably contains a single nymph.

The number of galls found on one leaf may be as high as 76, but 20 galls per leaf may be taken as an average.

Mere oviposition does not induce gall formation; therefore, the number of galls does not correspond with the number of eggs laid.

Emergence of the nymph from the gall.—A full-grown nymph places itself vertically in the gall with the tip of its abdomen close to the exit. This aperture, which has hitherto remained closed, begins to open, and by the time the nymph is ready to leave the gall, it has opened sufficiently wide to allow the insect to escape. The nymph leaves the gall walking backwards with abdomen foremost and crawls on to the flat surface of the leaf just beside the gall. Soon after its escape it moults and becomes adult.

Colouration of the newly emerged adult.—Head, thorax, and the first half of abdomen yellowish, dorsally the base of abdomen with a whitish-yellow horizontal stripe; eyes slaty-grey; ocelli luteous; sternum, underside of abdomen and the second half of abdomen above, stramineous; antennæ and legs translucent white; terminal setæ of antennæ dark or dark ochraceous and of unequal length. Apex of rostrum and tips of antennæ black. Wings hyaline. Veins yellowish except Cu_2 black.

After six hours the adult becomes "red or dark reddish brown" [Crawford, 1912], there being a good deal of variation in the degree of intensity of colouration.

Copulation and oviposition.—The adult insects congregate in fairly large numbers on young leaves where copulation takes place. Copulation takes place from side to side with the heads of the pair pointing in the same direction. When in coitus they move about the young leaves freely.

Soon after copulation the female starts oviposition. Eggs are laid singly on the underside of the young leaf (Plate XXXVI, fig. 7) along the midrib, on the margins of the leaf, or scattered on the lamina. Very rarely, however, eggs may be deposited on the upper side of the leaf.

The egg (Plate XXXVI, figs. 7e, 7es, 7et).—The egg is cylindrical and tapers abruptly towards each end (Plate XXXVI, figs. 7es, 7et). The basal end is rounded and produced into a stalk which is thrust into the tissue of the leaf, but the free distal end is acute and terminates in a fine bristle-like process (Plate XXXVI, fig. 7e). The egg is slightly curved (Plate XXXVI, fig. 7es) and along its convex side is a shallow longitudinal groove which marks the position

of the future slit along which the egg shell will split at the time of hatching (Plate XXXVI, fig. 7et). When freshly laid the egg is light yellow, but becomes darkish just before hatching. Empty egg shells remain attached to the leaf a long time after hatching.

Incubation period and the behaviour of the newly hatched nymph.—The egg stage lasts from three to five days during August (at Pusa—Bihar). The nymph on hatching crawls about on the surface of the leaf for some time and then settles down lying perfectly flat with the whole of the undersurface of its body pressed well against the surface of the leaf and the legs well spread out.

Nymphal colouration and wax production.—The nymphs of the first and the second stage are translucent with red eyes. In the third stage the general body colour becomes orange-yellow with the underside of the head and thorax white. The secretion of wax begins at this stage. The colour changes somewhat in the fourth stage and the wax production becomes more profuse. Head and thorax (with the exception of the wing-pads which are transparent) become yellowish and abdomen is orange-yellow with its tip covered with wax. In the fifth stage no change in colour takes place excepting the wing-pads which become yellowish, but during this instar wax production is at its maximum with the result that abdomen is completely covered with it and the rest of the body is lightly dusted with wax.

Moults.—Five moults are gone through before the winged stage is reached. Four moults take place within the gall and the fifth one outside on the leaf. If a gall is carefully opened all the nymphal skins of the respective instars can be easily found sticking to the inside of the gall.

Injury.—The insect causes injury to the young growing leaf by sucking its juices and by producing galls which interfere with its proper functioning. After the escape of the nymphs these galls become woody and black. The infested leaves are distorted and undersized.

Description of the nymphal instars of P. tuberculata *Craw.*

Fifth instar nymph (Plate XXXVI, figs. 5, 5a, 5ss, 5as, 5ts, 5t, 5tg, 5ca, 5p, 5m ; Plate XL, fig. 1).—Length on slide :—1·1—1·24 mm.

The nymph of Pauropsylline type with body flattened marginally fringed (except the basal portion of the margin of abdomen) with long and slender setæ borne on small tubercles, interspersed with a few short ones, all equally spaced.

Head short and broad. Dorsally, except along a median-longitudinal membranous streak, derm is heavily chitinized but ventrally membranous throughout. Eyes large, many-faceted. Antenna (Plate XXXVI, fig. 5a) ·36—·49 mm. long, tapering, bent backward passing over the middle of the eyes and during life

held against the sides of the head; corrugated except basally and apically where they are smooth, segmentation not clear although lightly stained areas divide each antenna into seven segments, of which the seventh segment is the longest. Segments 6th and 7th each with a ring-shaped sensorium (Plate XXXVI, fig. 5a. s). Apical half of 7th segment bears two long setæ, one on the lower side (Plate XXXVI, fig. 5as) and a longer one on the upper side (Plate XXXVI, fig. 5ss). (The position of these setæ is characteristic of every stage.)

Thorax membranous. Legs strong, stout, and chitinized and the nymph can walk with ease. Trochanter not formed. Each tibia with two long spines at apex on the underside and a shorter one on the upper side a little removed from the apex (Plate XXXVI, fig. 5ts). Tibio-tarsal articulation present (Plate XXXVI, fig. 5t). Tarsi unsegmented. A pair of small claws present. Pulvillus absent. Inserted dorsally on the apex of the tarsi just above the claws are two hollow golf-club-shaped setae (Plate XXXVI, fig. 5tg). Wing-pads large, heavily chitinized, not prominently standing out of the general contour of the body; humeral angle not produced cephalad, but shows a tendency in this direction, the outer margin of the wing-pads is in line with the outer margins of head and abdomen. This type of nymph I have called Pauropsylline type.

Abdomen flattened, and devoid of the marginal setæ on its basal portion, otherwise well supplied with setæ. Its apical fourth is heavily chitinized dorsally, but ventrally it is membranous excepting small chitinized areas round the spiracles. Ventrum of apical fourth of abdomen is clothed in minute points (Plate XXXVI, fig. 5m). Anal opening situated a little in front of the apex of the abdomen and enclosed within a circum-anal pore ring which is interrupted medially, both in front and behind (Plate XXXVI, fig. 5ca; Plate XL, fig. 1). Circum-anal pore ring slightly sinuous; its pores oval (Plate XXXVI, fig. 5p).

Fourth instar nymph (Plate XXXVI, figs. 4, 4a, 4ca, 4p).—Length on slide :— ·78—·87 mm.

General shape same as of the nymph of the fifth instar (Plate XXXVI, fig. 4); each antenna ·21 mm. long; wing-pads smaller and not overlapping; tibio-tarsal articulation lacking; circum-anal pore ring narrowly U-shaped, not sinuous (Plate XXXVI, fig. 4ca). The apical seta of each antenna reaches the tip of it (Plate XXXVI, fig. 4a. ss). The dorsum of head and thorax clothed in long, slender, scattered setæ; abdominal setæ as a whole longer than those of the fifth instar nymph.

Third instar nymph (Plate XXXVI, figs. 3, 3a, 3ca, 3p).—Length on slide :— ·53 mm.

Resembles the nymph of the fourth instar but differs in having each antenna ·05 mm. long; smaller though quite distinct wing-pads (Plate XXXVI, fig. 3);

smaller circum-anal pore ring (Plate XXXVI, fig. 3ca) with more or less circular pores (Plate XXXVI, fig. 3p); the apical seta passing the tip of each antenna (Plate XXXVI, fig. 3a. ss); body setæ longer.

Second instar nymph (Plate XXXVI, figs. 2, 2a, 2ca).—Length on slide:—·32 mm.

Head broadly rounded in front (Plate XXXVI, fig. 2); each antenna ·04 mm. long; apical seta of each antenna inserted just about the tip and largely passing it (Plate XXXVI, fig. 2a. ss); wing-pads rudimentary; body setæ fewer in number and V-shaped circum-anal pore ring (Plate XXXVI, fig. 2ca). In other respects it resembles the third instar nymph.

First instar nymph (Plate XXXVI, figs. 1, 1t, 1a, 1s).—Length on slide:—·24 mm.

Oblong, body fringed with lanceolate setæ (Plate XXXVI, fig. 1s). Head narrowly rounded in front; each antenna ·03 mm. long, apical seta of each antenna (Plate XXXVI, fig. 1a. ss) inserted at its tip; claws absent; pulvillus present, well developed, fan-shaped and petiolate (Plate XXXVI, fig. 1t); wing-pads and circum-anal pore ring lacking. Otherwise identical with the second instar nymph.

About the fifth stage nymph of *Leuronota michoacana* F. (Sub-family: TRIOZINÆ) Ferris [1928, 2] remarks: "departing from the typical triozine form in that the wing-pads are not produced anteriorly, but otherwise of this general type....." while Klyver [1930] describes the fifth stage nymph of *Aphalara calthæ* L. (Sub-family: LIVIINÆ) as "elongate and of typical psylliine form, that is, with the wing-pads projecting from the side of the body and not produced cephalad.....". From the figure of the fifth stage nymph of *Leuronota michoacana* F. given by Ferris it is clear that it approaches what I call the Pauropsylline type more closely rather than triozine type [cf. *Ceropsylla sideroxyli* Riley, Ferris 1923, p. 255], because the wing-pads are not produced cephalad and their lateral margins run in line with the lateral margins of head and abdomen. From the figure of *Aphalara calthæ* L. given by Klyver it is also clear that it belongs to my Pauropsylline type rather than Psylliine type in as much as the outer margins of the wing-pads run in line with the outer margins of head and abdomen. In a typical psylliine form [cf. *Psylla buxi* Linn. Ferris, 1926, p. 19] on the other hand the wing-pads are attached at an angle to the thoracic region.

PAUROPSYLLA DEPRESSA CRAW. (Sub-family: PAUROPSYLLINÆ).

(Plate XXXVIII.)

Material.—Nymphs were collected between 17th October to 7th December 1926 (Coll. K. A. R.), from galls on the leaves of *Ficus glomerata* Roxb., Imperial Agricultural Research Institute, Pusa, and on 9th November 1931 from Lahore

(Coll. K. G. Bhandari). The available material consists of the fifth, third, and first instars only.

The mature gall.—The nymphs make galls on the leaves of *Ficus glomerata* Roxb. These galls may occur singly or in clusters. The inner partition of each gall in a cluster remains quite intact.

Each gall is like a pointed sphere and mainly projects above the upper surface of the leaf. The top portion of the gall appears on the lower side of the leaf as a small elevation and is more or less pointed. The top of a gall is whitish-green in colour—sometimes with a reddish tinge—and contains the aperture of exit, *i.e.*, aperture is on the lower surface of the leaf.

Nymphal colouration and wax production.—The newly hatched nymph is semitransparent with the margins of the body fringed with waxy filaments. Once within the gall its colour changes to bright yellow and the filaments disappear. In subsequent stages no wax production takes place. After the first ecdysis head and thorax become whitish and the abdomen whitish yellow. In the third and the following instars the colour scheme is more or less identical and is as follows :—

Head and thorax including wing-pads pale tawny-brown ; a black spot situated about the centre of the base of each wing-pad ; eyes blood red ; legs, antennæ, whitish yellow ; dorsum of abdomen pale yellow with a double row of pale tawny dashes and spots contiguous caudad ; ventrum whitish yellow.

Moults.—Number of moults is five.

Injury.—A few galls do not affect the leaf adversely, but when numerous, as is usually the case, the leaf gets distorted, turns yellow, dries up and ultimately drops down.

Description of the nymphal instars of P. depressa *Craw.*

Fifth instar nymph (Plate XXXVIII, figs. 5d, 5da, 5db, 5dt, 5dp, 5dn, 5dg, 5dm, 5ds, 5dc ; Plate XL, fig. 2).—Length on slide :—2·1 mm.

The nymph is of the pauropsylline type with body oblong, integument thick, dorsally sparsely clothed in long setæ (Plate XXXVIII, fig. 5ds), ventrally and on the vertex of head in minute spine-like setæ (Plate XXXVIII, fig. 5dm).

Head rounded in front and not separated from the thorax by any distinct groove [cf. *Trioza fletcheri* Craw., fifth instar nymph (Plate XXXIX, fig. 5)]. Eyes large, many faceted and do not bulge laterad. Each antenna ·31 mm. long, tapering, three-segmented, strongly curved, and set ventrally well back from the apex of the head with the result that it hardly attains the middle of the eyes. It is very clearly imbricate, and is furnished with four sensoria (Plate

XXXVIII, figure 5da. s). Two small setæ near the apex of the last segment present.

Sternum is supplied with a very large number of sclerotic areas each one of which is mounted with palmate setæ (Plate XXXVIII, fig. 5db). Legs well-developed, trochanter absent, femora stout and not reaching the margins of the body. Tibio-tarsal articulation present (Plate XXXVIII, fig. 5dt); claws absent; pulvillus present, reniform and slightly petiolate (Plate XXXVIII, fig. 5dp). Apical half of tibiæ and the whole of tarsi (Plate XXXVIII, fig. 5dt) clothed in thorn-like setæ (Plate XXXVIII, fig. 5dg) which are arranged in transverse rows on the tarsi. On the dorsum of each tarsus, just above the pulvillus, there is a single slightly curved, golf-club-shaped seta pointed at its end (Plate XXXVIII, fig. 5dn). Wing-pads not produced cephalad, but at the same time their humeral angle slightly bulging and their outer margins run in line with the margins of head and abdomen.

Anus set a little in front of the apex on the ventral side; circum-anal pore ring (Plate XXXVIII, fig. 5dca; Plate XL, fig. 2), made up of slit-like pores, is more or less rounded in outline and is interrupted both anteriorly and posteriorly. Circum-anal pore ring encloses another ring of minute circular pores (Plate XXXVIII, fig. 5dca).

Third instar nymph.—Length on slide :—·62 mm.

In general characters resembles the fifth instar nymph but differs as follows :—Each antenna ·08 mm. long, slightly curved, with segmentation ill-defined. Tibio-tarsal articulation lacking; body clothed in long setæ which give the insect a shaggy appearance.

First instar nymph.—Length on slide :—·24 mm.

Body oblong, margined with hollow tube-like setæ widely spaced and set on tubercles. These setæ disappear in subsequent stages. Each antenna ·04 mm. long, straight, and imbricate with segmentation obscure. Tip of each antenna armed with two setæ of unequal length. Wing-pads absent. Body setæ are fewer in number. In other respects it resembles the nymph of the third instar.

Tenaphalara elongata Craw. (Sub-family : carsidarinæ).

(Plate XXXVII.)

Material.—Eggs and nymphs were collected between 12th December 1926 to 24th January 1927 (Coll. K. A. R.) from the underside of the leaves of *Bombax malabaricum* DC., Imperial Agricultural Research Institute, Pusa.

Oviposition.—The eggs are laid singly in the tissue of the leaf of *Bombax malabaricum* DC., their presence being indicated by a slight excrescence and by the dirty-white colouration of the surrounding tissue. The eggs are elongate-oval and are neither stalked nor furnished with a bristle. On hatching the nymphs crawl about on the under surface of the leaf.

Nymphal colouration and wax-production.—The first, second and third instar nymphs are generally brownish yellow, but change to green in the fourth and fifth instars, in addition there is a longitudinal median red line running from the tip of the head to the caudal extremity. This is particularly well-defined in the nymphs of the fifth instar. There are also two black dots situated one on either side of the red stripe about the bases of the hind wing-pads.

To start with wax-production is very slight and in the first instar nymph it forms a coating of the stalked bubble of honey-dew, but as the nymph grows and advances in age wax-production becomes more profuse, until in the fifth instar it is at its maximum and is given out from the circular pores of the chitinized area in the form of very long and straight waxy filaments with a stalked bubble of honey-dew coated with wax sticking out from the circum-anal pore ring (Plate XXXVII, fig. 5e). These waxy filaments are as long or even longer than the entire nymphal body and they, together with the accumulated dirt particles and the cast off nymphal skins, impart a very characteristic appearance to the nymphs. These waxy filaments are easily detachable.

Habits of the nymphs.—The nymphs are free living and during December and January (Pusa—Bihar) all stages from the egg to the adult are to be found on the same leaf. Nymphs of all instars remain congregated on the underside of the infested leaf on either side of the mid-vein. They are very active and agile, and when disturbed run about the leaf with great speed.

Injury.—According to Fletcher [1917] "the attack may be severe but of less importance as the leaves fall off the trees in another couple of months' time" (from December).

Description of the nymphal instars of T. elongata *Craw.*

Fifth instar nymph (Plate XXXVII, fig. 5, 5a, 5as, 5l, 5t, 5ca, 5c, 5e; Plate XL, fig. 3).—Length on slide :—1·7—2·1 mm.

The nymphs of typical psylliine type with body elongate not flattened, sides of the body sub-parallel, wing-pads not projecting cephalad at the humeral angle but stand well away from the sides of the body.

Head large, rounded in front, truncate behind and distinctly marked off from the thorax. Derm of head is heavily chitinized dorsally, but ventrally, excepting a small squarish chitinized plate about the base of each antenna, membranous

and clothed in short setæ which are scattered irregularly. Eyes prominent, not projecting laterad. Each antenna 1·3 mm. long, segmentation distinct, ten-segmented; first segment short and stout; second segment short and cylindrical, rest long and slender; first two segments furnished with short, slender setæ, third to ninth segments apically bordered with short, slender, setæ, apex of terminal segment furnished with two setæ situated as indicated in the figure (Plate XXXVII, fig. 5a). Apical portion of the third segment, the entire fourth and successive segments clearly imbricate, segments two to nine each carry one sensorium situated apically (Plate XXXVII, fig. 5as).

Thorax, excepting the heavily chitinized wing-pads, membranous both dorsally and ventrally. Legs long and beset with short stiff setæ. Trochanter absent, femora stout and passing the margins of the body. Tibio-tarsal articulation quite distinct (Plate XXXVII, fig. 5t). Claws present, but pulvillus absent. The two golf-club-shaped setæ present (Plate XXXVII, fig. 5tg). Wing-pads over-lapping. Outer margins of the front wing-pads and posterior margin of the hind wing-pads fringed with widely spaced short but stout setæ borne on tubercles.

Abdomen 1·1 mm. in length and longer than the head and the thorax put together, slightly narrowed at base gradually swells out to its apical fourth and then obliquely dips and terminates in an ill-defined process which carries the anus. The circum-anal pore ring, interrupted medially both anteriorly and posteriorly, consists of a very large number of more or less circular pores which are arranged in a comma-shaped manner (Plate XXXVII, fig. ca). The inner ring of minute pores is absent. In addition there are chitinized areas perforated with circular pores (Plate XXXVII, fig. 5c; Plate XL, fig. 3), located in three definite groups along the dorso-lateral and latero-ventral margins of the apical portions of the body, each group being continuous both above and beneath (Plate XXXVII, fig. 5). It is through these pores that the long waxy filaments described above are given out. Apical fourth of abdomen marginally fringed with lanceolate setæ (Plate XXXVII, fig. 5l) which are widely spaced and interspersed with simple setæ. The derm excepting the last mentioned chitinized areas membranous both dorsally and ventrally.

Fourth instar nymph (Plate XXXVII, figs. 4, 4t.).—Length on slide :—1·3 mm.

In general characters identical with the fifth instar nymph but differs as follows: Each antenna eight segmented, ·84 mm. long with the fourth segment longest (·12 mm.), with only the last four segments imbricate. Chitinized squarish piate at the bases of each antenna absent. Wing-pads are smaller and slightly over-lapping. Femora comparatively stouter being on an average ·16 mm. thick at the thickest portion. Tibio-tarsal articulation lacking (Plate XXXVII, fig. 4t).

Third instar nymph (Plate XXXVII, fig. 3.).—Length on slide :—·8 mm.

Each antenna six-segmented, ·45 mm. long, last three segments faintly imbricate. Head anteriorly truncate, laterally broadly rounded, frontal margin of the head between the bases of antennæ supplied with short stiff setæ arranged in three transverse lines, one behind the other. Area round about proboscis heavily chitinized. Eyes quite prominent and bulging laterad. Wing-pads small and not overlapping.

In other respects it resembles the fourth instar nymph.

Second instar nymph (Plate XXXVII, figs. 2, 2a, 2pd, 2ca.).—Length on slide :—·6 mm.

Each antenna (Plate XXXVII, fig. 2a) three segmented, ·22 mm. long, third segment longest being about ·17 mm. with two sensoria situated at about its middle, one on the dorsal and the other on the ventral side. Wing-pads appear as slight swellings from the sides of mesothorax and metathorax. Circum-anal pore ring made up of a single row of pores (Plate XXXVII, fig. 2ca) and the circular pores of the chitinized area arranged in two groups only (Plate XXXVII, fig. 2). Slightly above the upper group the abdominal wall swells out to form a pouch-like dilatation which is mounted by a seta (Plate XXXVII, fig. 2pd).

In other respects it resembles the nymph of the third instar.

First instar nymph.—Length on slide :—·45 mm.

Each antenna two jointed, ·09 mm. long, second joint longest being ·08 mm. Ventrum of the head is beset with short, stiff setæ.

The condition of the abdominal portion of the specimen does not permit of a definite statement being made about the circum-anal pore ring and the circular pores.

ARYTAINA PUNCTIPENNIS CRAW. (Sub-family : PSYLLINÆ).

(Plate XXXVIII.)

Material.—Nymphs were collected in December 1926, (Coll. K. A. R.) from Indigo (*Indigofera*), Imperial Agricultural Research Institute, Pusa.

Oviposition and the egg.—According to Lefroy [1913] "eggs are laid singly, stuck to the plant; they are laid near the tip of the shoot if there is room, either on the stem itself, or on the leaf-stalks or the leaflets. Each egg is cylindrical, tapering to each end, one end a little broader than the other; when first laid they are very pale yellow, almost white; in two days they become deep black".

Injury.—The nymphs are free living, they remain congregated on the stem and side shoots of the indigo plant, and extract sap with the result that in "the Java tip curls into a very compact hard knot owing to the thick stem and crowded leaves" while "the Sumatrana with their leaves well spread out curl into less compact masses" [Lefroy, 1913].

Description of the nymphal instars of A. punctipennis Craw.

Fifth instar nymph (Plate XXXVIII, fig. 5, 5a, 5b, 5t, 5tg, 5ts, 5s, 5ca, 5cp, Plate XL, fig. 4).—Length on slide:—1·5 mm.

General form typical psylliine, marginally fringed with setæ of three varieties (1) sharply pointed sectasetæ, (2) lanceolate setæ, and (3) lanceolate setæ with jagged tips (Plate XXXVIII, fig. 5b. sc. l. j.), interspersed with each other, all set on tubercles.

Head distinct, anteriorly rounded, with derm chitinized both dorsally and ventrally. Eyes large semi-circular, and project slightly from the sides of the head. Each antenna (Plate XXXVIII, fig. 5a) eight-segmented, ·51 mm. long, third joint longest being about ·11 mm. long; segments four to eight imbricate. Tip of the last segment bears two short setæ which are basally convergent but apically divergent. Segments three to seven, each bears a sensorium on the lower side of its apex, but on the eighth segment sensorium is situated about the middle (Plate XXXVIII, fig. 5a. s).

Thorax largely taken up by wing-pads. Legs quite thickly beset with slender setæ. Trochanter present. Femora of the front and the middle legs passing and those of the hind legs just attaining the margins of the body. Tibio-tarsal articulation present (Plate XXXVIII, fig. 5t); claws present but pulvillus absent. On the upper side of each tarsus (Plate XXXVIII, fig. 5t), just above the claws, two hollow golf-club-shaped setæ present (Plate XXXVIII, fig. 5tg). Wing-pads large, stand well out of the general contour of the body; both legs and wing-pads chitinized.

Abdomen flattened. Apical half and a few scattered areas chitinized dorsally but ventrally membranous except the chitinized apical fourth and the chitinized areas round the spiracles (Plate XXXVIII, fig. 5). Ventrum beset with four types of setæ.

i. simple setæ (Plate XXXVIII, fig. 5s),
ii. short thorn-like setæ (Plate XXXVIII, fig. 5ts),
iii. lanceolate setæ (Plate XXXVIII, fig. 5b. l),
iv. lanceolate setæ with jagged tips (Plate XXXVIII, fig. 5b.j).

Anal opening triangular and a little in front of the tip of the abdomen. Circum-anal pore ring (Plate XXXVIII, fig. 5ca; Plate XL, fig. 4) continuous, broad bean-shaped, and is made up of oval pores enclosing within another ring of circular pores. Slightly in front of the tip of the abdomen one on either side of the circum-anal pore ring there are circular pores arranged in the form of a semi-circle and each of these encloses another ring of minute circular pores within (Plate XXXVIII, fig. 5cp).

Fourth instar nymph (Plate XXXVIII, fig. 4).—Length on slide:—·83 mm.

It resembles the fifth instar nymph but differs as follows :—Each antenna are ·31 mm. long, five-segmented, segment fifth longest, being ·12 mm. and segment third is second longest, being ·08 mm. Trochancter absent. Tibio-tarsal articulation lacking.

Third instar nymph (Plate XXXVIII, figs. 3, 3a, 3ca, 3cp).—Length on slide :—·58 mm.

Resembles the fourth instar nymph but differs as follows :—Smaller ; each antenna (Plate XXXVIII, fig. 3a) three-segmented, ·16 mm. long, third segment longest, being ·12 mm. with two sensoria on its lower side, one medial, other post-medial (Plate XXXVIII, fig. 3a. s). Wing-pads smaller and do not overlap.

Arrangement of circular pores pyriform (Plate XXXVIII, fig. 3cp). Chitinized areas as indicated in the figure (Plate XXXVIII, fig. 3).

TRIOZA FLETCHERI CRAW. (Sub-family : TRIOZINÆ.)

(Plate XXXIX.)

Material.—Eggs and nymphs were collected between 17th of November 1926 to 26th January 1927, (Coll. K. A. R.) from the leaves of *Trewia sp.*, Imperial Agricultural Research Institute, Pusa.

The mature gall, (Plate XXXIX, figs. 6, 6a).—Galls generally occur in groups which are very irregular in outline. An individual gall is a flask-shaped structure. A larger portion of the gall projects towards the upper side of the leaf and due to swelling up of the leaf-veins is very much corrugated and rough (Plate XXXIX, fig. 6). The upper portion which shows as a slight depression towards the lower-side of the leaf is smooth and is clothed in thick whitish pubescence. It is this upper portion that contains a longitudinal slit which towards the termination of the nymphal period, opens out wide enough to allow the escape of the full grown nymph. The interior of the gall is smooth.

The egg (Plate XXXIX, fig. 7).—The eggs are laid singly on the leaves of *Trewia sp.* Each egg is anchored by means of a stalk-like process situated at its basal extremity. After eclosion their whitish shells remain attached to the leaves for a considerably long time.

The egg is thick and blunt at its basal portion but tapers towards its distal extremity where on its convex side it is strongly curved and terminates in a twisted bristle.

Nymphal colouration and wax production.—The nymph is whitish in the first four instars but whitish-yellow in the fifth instar. Eyes, however, remain brick-red in all instars. Wax-production is very profuse in all stages. During life sectasetæ

are mounted with long waxy tubes (Plate XXXIX, fig. 5b), while the circum-anal pore ring produces waxy material which surrounds the honey-dew globule. The floor of the gall is always littered with these honey-dew globules covered with wax.

Moults.—Four moults take place within the gall and the fifth one outside on the leaf.

Injury.—After the escape of the nymph, galls become woody.

Description of the nymphal instars of T. fletcheri *Craw.*

Fifth instar nymph (Plate XXXIX, figs. 5, 5a, 5ss, 5b, 5t, 5d, 5v, 5ca, 5p; Plate XL, fig. 5).—Length on slide:—1·66 mm. including sectasetæ; and 1·61 mm. excluding sectasetæ.

The nymph of the triozine type with body oval marginally fringed with closely spaced sectasetæ mounted on tubercles (Plate XXXIX, fig. 5ss), and with minute points (Plate XXXIX, fig. 5d).

Head distinct, rounded in front and on the sides, posteriorly truncate and marked off from the thorax by means of a transverse membranous groove. Derm of head heavily chitinized dorsally but ventrally smooth and thickly beset with minute points (Plate XXXIX, fig. 5d). Eyes small, globular, and many facetted. Each antenna (Plate XXXIX, fig. 5a) five jointed, situated on the ventrum of the head well back from its margins. Each antenna ·24 mm. long, first segment longest (·11 mm.) tapering and just reaching the margins of head, second, third, and the fourth segments small, ring-shaped; fifth segment second longest, being ·09 mm. long. Six sensoria (Plate XXXIX, fig. 5a. s=sensorium) on the lower side of each antenna are situated as follows: two on the first, one on the second, one on the tourth and two on the fifth. Last segment imbricate and furnished with two setæ as shown in the figure.

Derm of thorax dorsally chitinized but ventrally membranous. Just below each hind coxa there is a papilla (Plate XXXIX, fig. 5v). All femora just reach margins of the body. Trochanter lacking, tibio-tarsal articulation present (Plate XXXIX, fig. 5t); tarsi without claws, pulvillus curious-looking structure being in the form of a circular pad which is useful in locomotion. There is a single golf-club-shaped seta present on the dorsal side of each tarsus (Plate XXXIX, fig. 5tg). Just about the tibio-tarsal articulation there are on the lower aspect two long setae reaching about the middle of tarsi (Plate XXXIX, fig. 5t. s'). Wing-pads chitinized, their humeral angle produced forward to about the middle of eyes.

Derm of abdomen, excepting for small membranous areas at the base, heavily chitinized dorsally but ventrally membranous throughout and thickly beset with minute points (Plate XXXIX, fig. 5d). Anus, set well back from the tip of the abdomen, is surrounded with a continuous circum-anal pore ring which is made up

of oval pores (Plate XXXIX, fig. 5p) enclosing within another row of minute pores (Plate XXXIX, fig. 5ca; Plate XL, fig. 5).

Fourth instar nymph (Plate XXXIX, figs. 4, 4a).—Length on slide :—·92 mm.

In general characters it resembles the fifth instar nymph but differs as follows :—

Each antenna (Plate XXXIX, fig. 4a) ·09 mm. long, four-segmented, segment second with a sensorium on its ventrum and the fourth segment longest with two sensoria on its lower side. Each antenna slightly pass the margins of head. Tibiotarsal articulation absent.

Third instar nymph (Plate XXXIX, fig. 3).—Length on slide :—·62 mm.

Resembles the last stage nymph but differs in having each antenna ·07 mm. long, three-segmented with two sensoria on the underside of the second and third segments and wing-pads smaller.

Second instar nymph (Plate XXXIX, fig. 2).—Length on slide :—·39 mm.

Each antenna ·04 mm. long, two segmented; wing-pads smaller but quite distinct. Papilla absent. In other respects it is identical with third instar nymph.

First instar nymph (Plate XXXIX, fig. 1).—Length on slide :—·24 mm.

Each antenna ·03 mm. long, ringed but proper segmentation not clear. Wing-pads represented by chitinous sclerites. Abdominal segments distinct and dorsally chitinized. In other characters it resembles second instar nymph.

NOTE :—According to Ferris [1925] sectasetæ "occur characteristically in the triozinae —" and "are apparently modified setæ and are associated with, if not responsible for, the formation of the slender waxen threads that are characteristic of the nymphs of many of this sub-family".

SUMMARY.

The classification on the family PSYLLIDÆ by Crawford and its nymphal types proposed by Ferris have been discussed and a new type has been added to the two proposed by Ferris.

A list of Psyllid nymphs so far fully described is given.

The nymphal forms of five Indian PSYLLIDÆ, *viz.* 1. *Pauropsylla tuberculata* Craw. 2. *Pauropsylla depressa* Craw., 3. *Tenaphalara elongate* Craw., 4. *Arytaina punctipennis*, Craw. and 5. *Trioza fletcheri* Craw. are described and figured.

The microscopic examination of the nymphal forms revealed a number of new structures for which special nomenclature had to be devised.

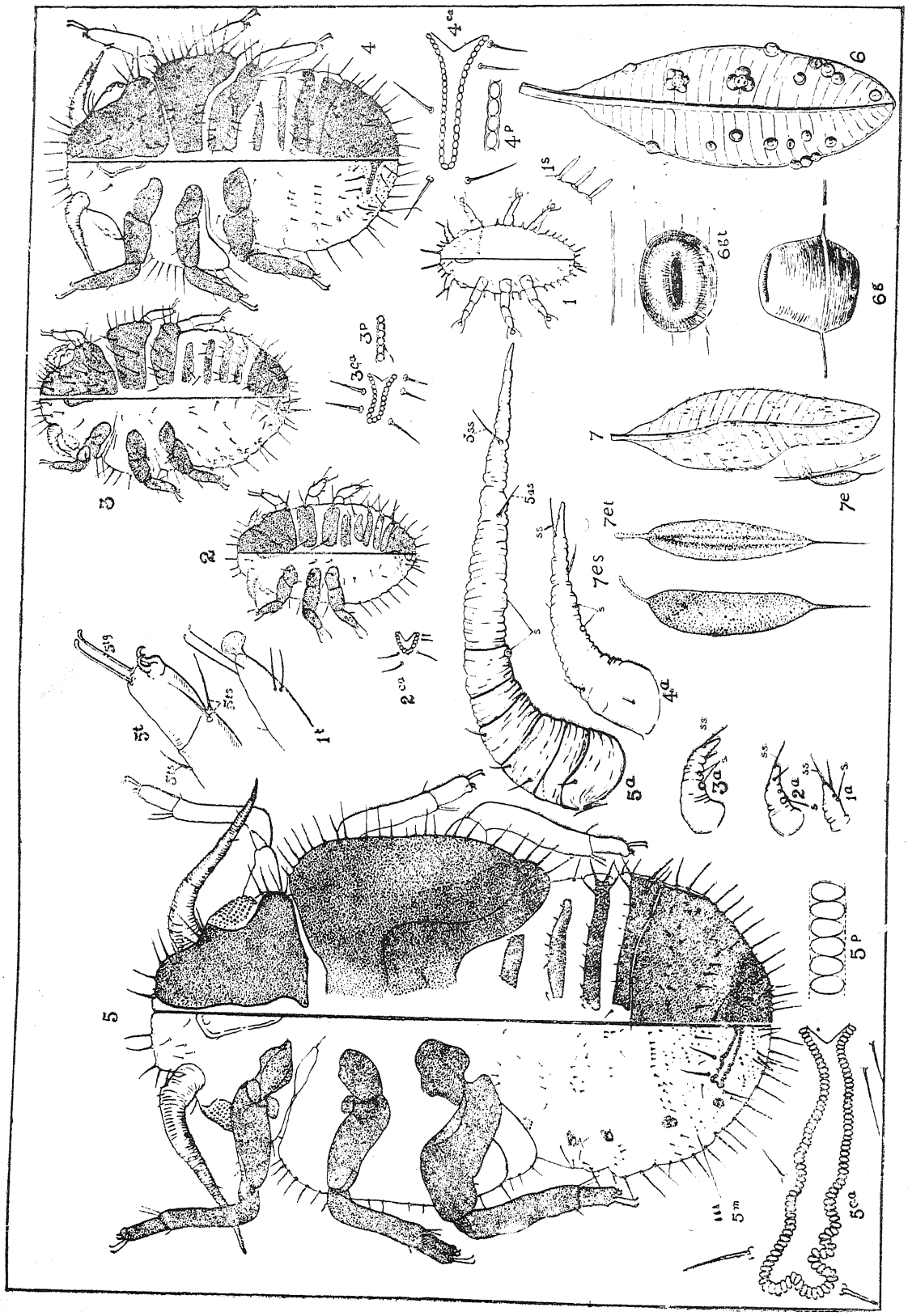
REFERENCES.

Afzal Husain & Dina Nath (1929). *Mem. Dept. Agric. Ind., Ent. Ser.* **10**, 5-17.

Buckton (1893). *Ind. Mus. Notes* **3**, 13-14; 91-92.

Buckton (1900). *Ind. Mus. Notes* **5**, 35-36.

Crawford (1912). *Rec. Ind. Mus.* **7**, 419-435.



Immature stages of *Pauropsylla tuberculata* Craw.
(*For explanation, please see p. 376.*)

Crawford (1914). *U. S. Nat. Mus. Bul.* **85**, 1-186.
„ (1919). *Philippine J. of Sci.* **15**, 2, 139-205.
„ (1924). *Rec. Ind. Mus.* **26**, 615-616.
Eerris & Persis Hyatt. (1923). *Can. Ent.*, **55**, 88-92.
Ferris (1923). „ „ **55**, 250-256.
——— (1925). „ „ **57**, 46-50.
——— (1926). „ „ **58**, 13-20.
——— (1928,1). „ „ **60**, 109-117.
——— (1928,2). „ „ **60**, 240-245.
Fletcher, T. Bainbrigge (1914). Some South Indian Insects, 498.
——— (1917). *Rept. Proc. Second Ent. Mtg., Pusa*, 131.
Grove & Gosh (1914). *Mem. Dept. Agric. Ind., Ent. Ser.* **4**, 329-357.
Klyver (1930). *Can. Ent.*, **62**, 167-175.
Lefroy (1909). Indian Insect Life, 1, 742-743.
——— (1913). *Agric. J. Ind.* **8**, 1-26.
Ramakrishna Ayyar (1919). *Rept. Proc. Third Ent. Mtg., Pusa*, **3**, 1030-1031.

Explanation of Plate XXXVI.

Immature stages of ***Pauropsylla tuberculata*** Craw.

1.—First instar nymph.
1a.—Antenna of first instar nymph showing position of the two setæ. s. sensorium. ss. apical seta.
1s.—Margin of body of first instar nymph showing enlarged lanceolate setæ.
1t.—Tarsus of first instar nymph showing golf-club-shaped setæ and fan-shaped pulvillus.
2.—Second instar nymph.
2a.—Antenna of second instar nymph. ss. apical seta.
2ca.—Circum-anal pore ring of second instar nymph.
3.—Third instar nymph.
3a.—Antenna of third instar nymph. ss. apical seta.
3ca.—Circum-anal pore ring of third instar nymph.
3p.—Types of pores of circum-anal pore ring.
4.—Fourth instar nymph.
4a.—Antenna of fourth instar nymph. ss. apical seta.
4ca.—Circum-anal pore ring of fourth instar nymph.
4p.—Types of pores of circum-anal pore ring (magnified).
5.—Fifth instar nymph.
5a.—Antenna of fifth instar nymph. s. sensorium { 5as. seta of lower side. / 5ss. „ „ upper side.
5t.—Terminal portion of the leg of the fifth instar nymph showing tibial setæ (5ts); and claws.
5tg.—Golf-club-shaped setæ.
5ca.—Circum-anal pore ring of fifth instar nymph.
5p.—Types of pores of circum-anal pore ring (magnified).
5m.—Minute points.
6.—Under surface of leaf of *Alstonia scholaris* showing galls.
6g.—The gall.
6gt.—Top of gall showing aperture of exit.

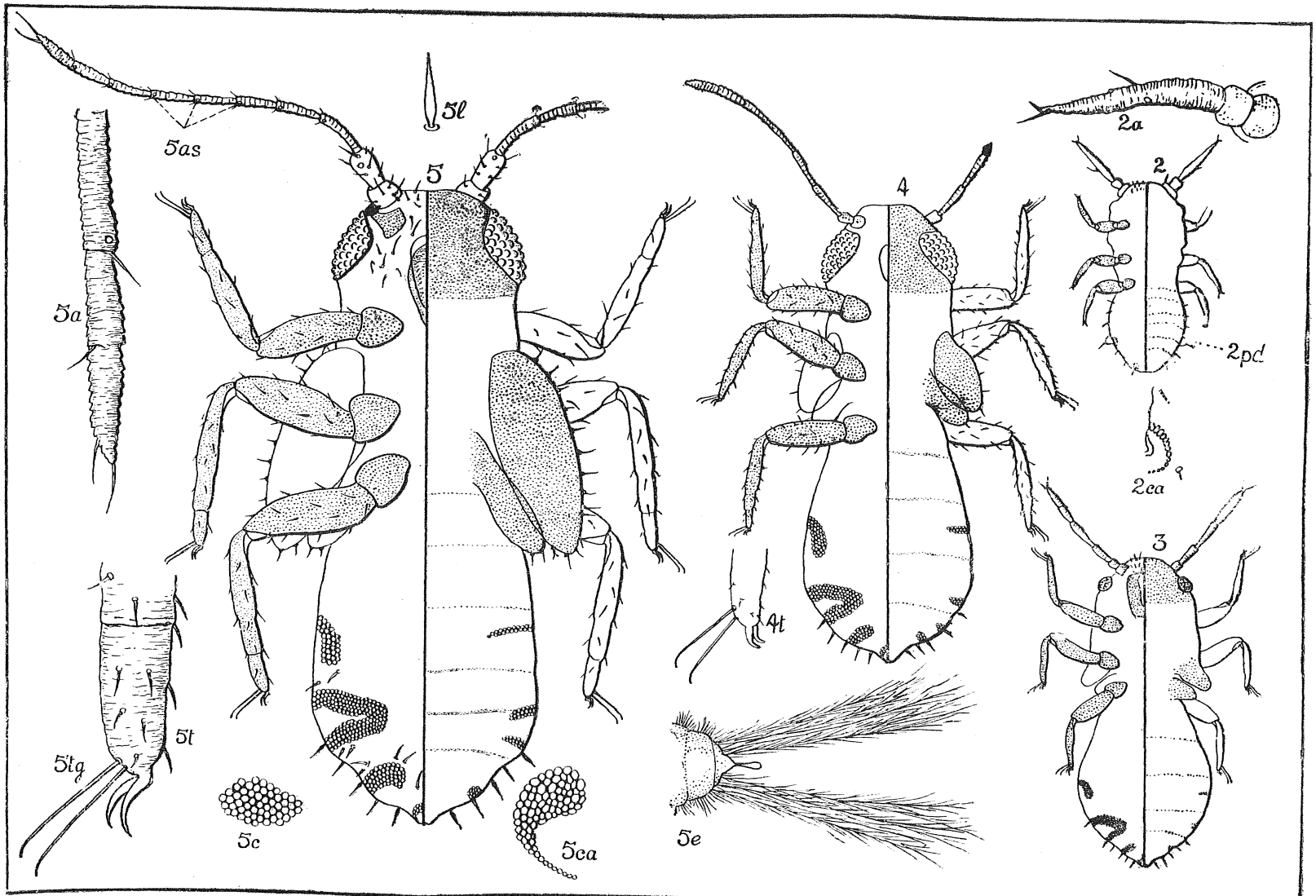
7.—Eggs on young leaf of *Alstonia scholaris*.
7e.—Edge of leaf showing attachment of egg.
7es.—Egg—front view.
7et.—Egg—side view—showing longitudinal groove.

Explanation of Plate XXXVII.

Immature stages of *Tenaphalara elongata* Craw.
2.—Second instar nymph.
2a.—Antenna of second instar nymph.
2ca.—Circum-anal pore ring of second instar nymph.
2pd.—Pouch-like dilatation of second instar nymph.
3.—Third instar nymph.
4.—Fourth instar nymph.
4t.—Tarsus of fourth instar nymph showing golf-club-shaped setæ and claws.
5.—Fifth instar nymph.
5a.—Antenna of fifth instar nymph.
5as.—Sensoria on the antenna of fifth instar nymph.
5t.—Tarsus of fifth instar nymph showing golf-club-shaped setæ (5tg); and claws.
5ca.—Circum-anal pore ring of fifth instar nymph.
5c.—Circular pores of the chitinized areas of the fifth instar nymph.
5l.—Lanceolate seta (magnified).
5e.—Tip of abdomen of fifth instar nymph showing long waxy filaments with the stalked bubble.

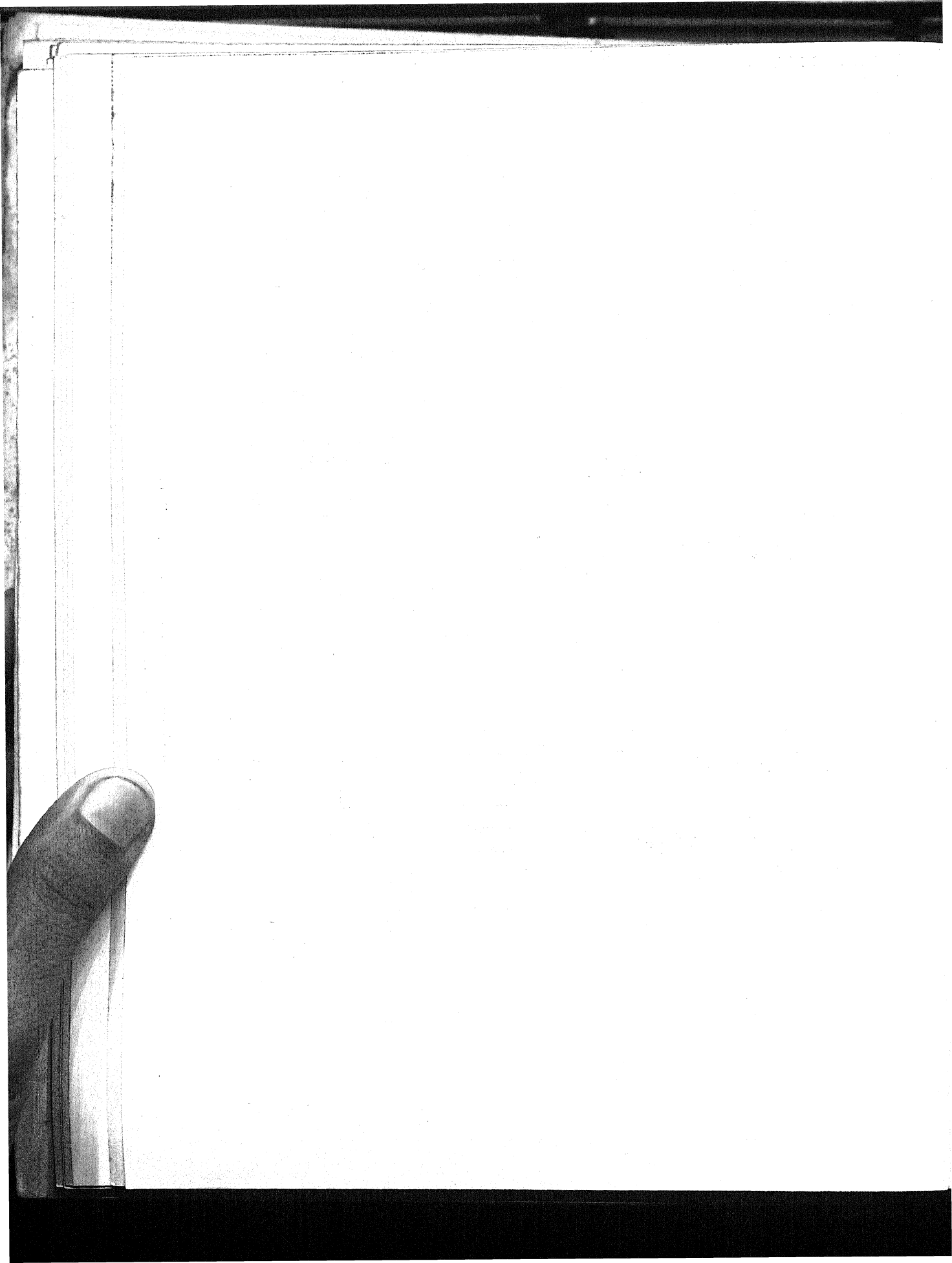
Explanation of Plate XXXVIII.

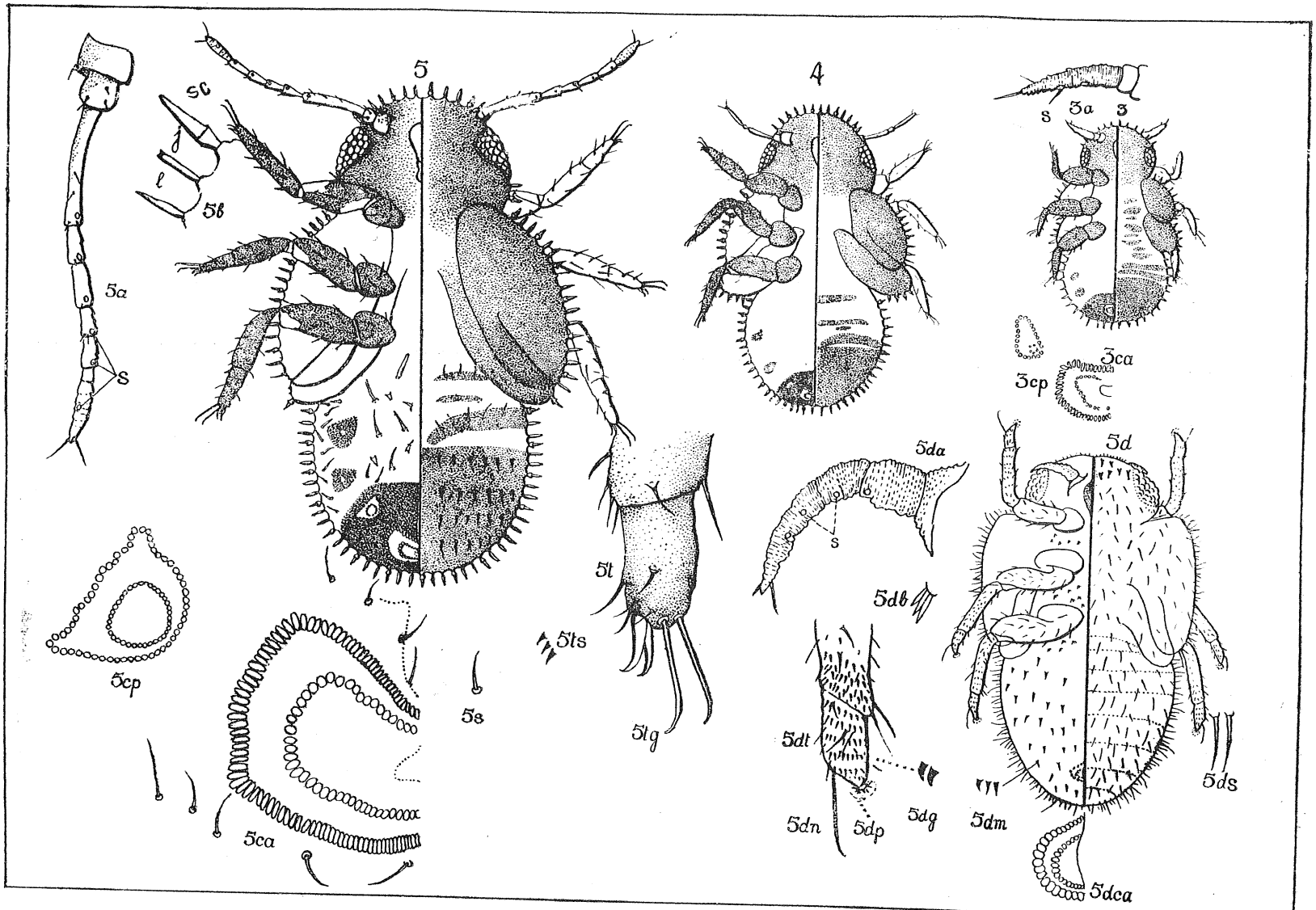
Immature stages of *Arytaina punctipennis* Craw. & *Pauropsylla depressa* Craw.
3.—Third instar nymph of *Arytaina punctipennis* Craw.
3a.—Antenna of third instar nymph of *Arytaina punctipennis* Craw.
3ca.—Circum-anal pore ring of third instar nymph of *Arytaina punctipennis* Craw.
4.—Fourth instar nymph of *Arytaina punctipennis* Craw.
5.—Fifth instar nymph of *Arytaina punctipennis* Craw.
5a.—Antenna of 5.
5b.—Margin of body of 5, showing the three types of setæ.
5ts.—Thorn-like setæ from the ventrum of abdomen of 5.
5s.—Simple setæ from the ventrum of abdomen of 5.
5t.—Tarsus of 5, showing golf-club-shaped setæ (*5tg*); and claws.
5ca.—Double circum-anal pore ring of 5.
5cp.—Circular pores of 5.
5d.—Fifth instar nymph of *Pauropsylla depressa* Craw.
5da.—Antenna of 5d. s. sensorium.
5dt.—Terminal portion of the leg of 5d. showing pulvillus (5dp); golf-club-shaped seta (5dn); and thorn-like setæ (5dg).
5db.—Palmate setæ of 5d.
5ds.—Simple setæ from dorsum of 5d.
5dm.—Spine-like setæ from ventrum of abdomen of 5d.
5dca—Double circum-anal pore ring of 5d.



Immature stages of *Tenaphalara elongata* Craw.

(*For explanation please see p. 376.*)





Immature stages of *Arytaina punctipennis* Craw. And *Paurocsylla depressa* Craw.
(*For explanation please see p. 376.*)

PLATE XL



Fig. 1.



Fig. 2.

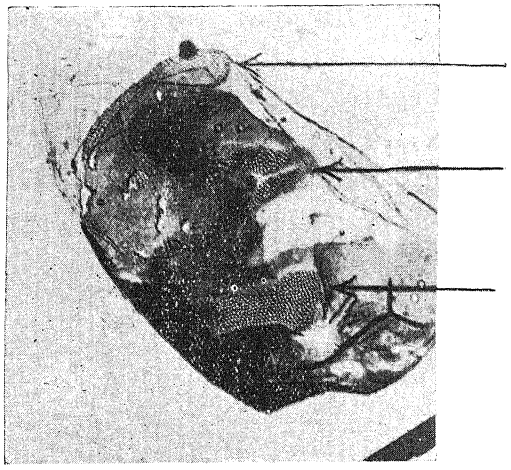


Fig. 3.

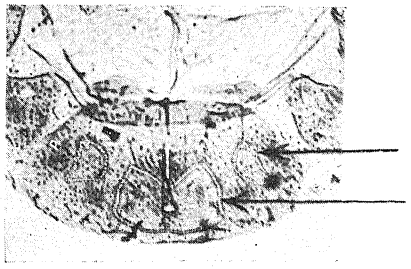


Fig. 4.

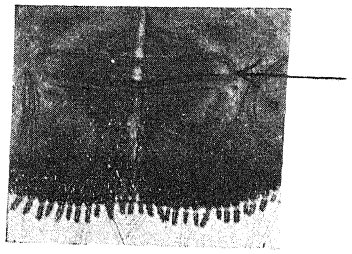
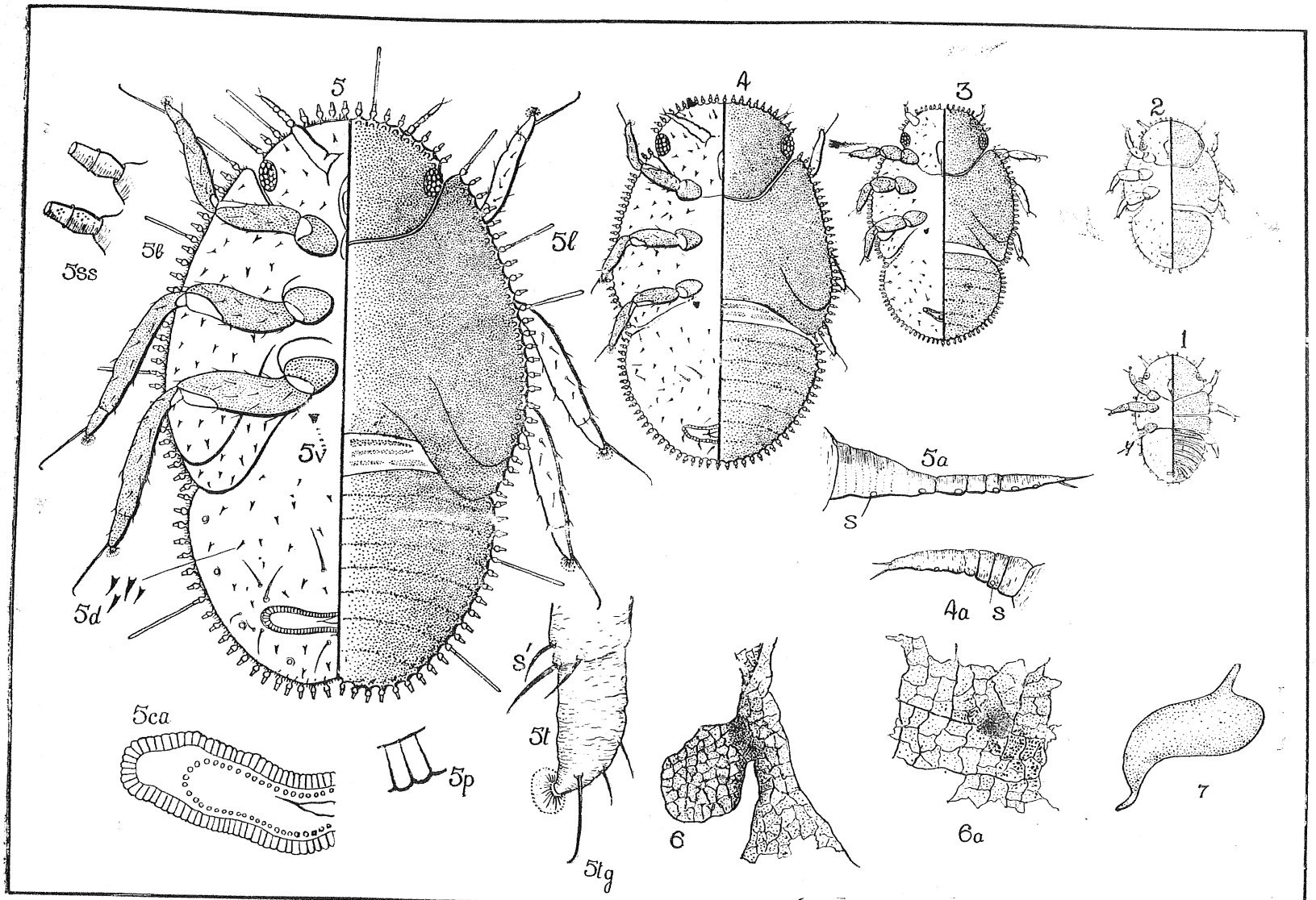


Fig. 5.

(*For explanation please see page* 377).



Immature stages of *Trioza fletcheri* Craw.
(*For explanation please see p. 376.*)

Explanation of Plate XXXIX.

Immature stages of *Trioza fletcheri* Craw.

1.—First instar nymph.
2.—Second instar nymph.
3.—Third instar nymph.
4.—Fourth instar nymph.
4a.—Antenna of fourth instar nymph, s. sensorium.
5.—Fifth instar nymph.
5a.—Antenna of fifth instar nymph. s. sensorium.
5ss.—Margin of body of fifth instar nymph showing enlarged sectasetæ.
5b.—Waxy tubes mounted on sectasetæ.
5t.—Tarsus of fifth instar nymph showing golf-club-shaped seta (*5tg*); and pulvillus.
5d.—Minute points (magnified).
5v.—Papilla.
5ca.—Anal opening with double circum-anal pore ring.
5p.—Types of pores of outer circum-anal pore ring.
6.—The gall——side view.
6a.—Upper surface of a leaf showing top portion of gall.
7.—Egg.

Explanation of Plate XL.

(Arrows point to the specified structures.)

Fig. 1.—Photograph of the circum-anal pore ring of the fifth instar nymph of *Pauropsylla tuberculata* Craw.

Fig. 2.—Photograph of the circum-anal pore ring of the fourth instar nymph of *Pauropsylla depressa* Craw.

Fig. 3.—Photograph of the circular pores of the abdominal chitinized areas of the fifth instar nymph of *Tenephalara elongata* Craw.

Fig. 4.—Photograph of the circum-anal pore ring and the semi-circularly arranged pores of the fifth instar nymph of *Arytaina punctipennis* Craw.

(Inner pores are visible in either case.)

Fig. 5.—Photograph of the circum-anal pore ring of the fifth instar nymph of *Trioza fletcheri* Craw.

EFFECT OF MOSAIC ON THE TONNAGE AND THE JUICE OF SUGARCANE IN PUSA, PART II.

BY

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(Received for publication on the 4th April 1932).

In continuation of last year's experiment recorded in this Journal * thirty-two plots each 5 by 66 yards were laid down in adjacent pairs half of them with mosaic-free and half with mosaic cane in the order mosaic-free, mosaic, mosaic, mosaic-free, and so on.

The area was good, even, heavy land suitable for growing sugarcane and its parts had been subject to the same field rotation in previous years. Immediately before this year the crop it held was sugarcane. The planting was done on the 26th and 27th February 1931 under my direction and the cultivation by the Imperial Agriculturist according to the usual practice on the farm. Castor cake at the rate of 25 maunds per acre was given at the time of planting. Planting was eye to eye and the shoots came up freely on all plots.

(*a*) *Mosaic cane.*—The sets were cut from canes whose leaves showed mosaic markings at the time of harvest, so this cane was definitely known to be mosaic-infected.

(*b*) *Mosaic-free cane.*—The sets were cut from cane that, from frequent observations during the growing season, was known to be entirely free from mosaic disease and at the time of harvest had no mosaic markings on the leaves. Thus the cane was definitely known to be mosaic-free when planted.

During the season 1931-32 a small amount of infection spread to the mosaic-free plots and 22 clumps were found to have the disease, four plots having one clump each, five having two each and one having eight. Such small numbers would have an effect quite inappreciable in the weighments of the plots. This small spread to healthy cane, in plots in such close juxtaposition to mosaic-infected cane as these are, indicates how few and how inactive insects were this season in spreading the disease.

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On the 15th April and the 14th May the number of shoots was counted and the percentages calculated on the number of buds on the sets planted taking three buds to a set. The percentage germination after six weeks and ten weeks is as follows :—

TABLE I.

Percentage germination.

Plot No.																		Mean
	F*	1	4	5	8	9	12	13	16	17	20	21	24	25	28	29	32	
	M*	2	3	6	7	10	11	14	15	18	19	22	23	26	27	30	31	
15-4-31	F	75	69	73	70	70	60	65	61	59	67	56	57	48	53	50	55	62
	M	58	54	48	47	46	49	54	51	51	48	48	51	58	50	53	51	51
14-5-31	F	81	88	100	86	86	75	67	78	59	75	84	61	53	65	53	61	73
	M	83	67	84	67	72	61	56	52	52	50	51	71	71	63	67	75	65

After the two periods the means of the mosaic plots were 11 and 8 per cent. below those of the mosaic-free plots respectively, but there is no correlation between the germination and the yield of the plots. The co-efficient of correlation works out in each case to a value which has no statistical significance.

From the middle of April the cane was attacked by the shoot-borer, *Scirpophaga nivella*. On the 14th of May and of June the bored shoots were counted and removed, after which very few other shoots became bored. The percentages of shoots bored in each plot is given in Table II. The high infection of *Scirpophaga nivella* early in the season was, I think, due to the fact that stubble of an adjacent field in which sugarcane was grown in the previous season was not thoroughly cleaned of stumps. Scirpophaga was found on the volunteer shoots that came up in March and April and probably passed from them to the cane in this experiment, thus indicating that one of the most important items in good cane cultivation with a view to reduce borer attack is early and clean cultivation of the previous year's

* F indicates mosaic-free plots and M mosaic-infected plots.

cane-stubble. That this can be done is shown by the fact that not one volunteer plant came up in these experimental plots though cane followed cane.

TABLE II.

Percentage of borer attack in May and June.

*F	M*	F			M		
Plots		May	June	Total	May	June	Total
1	2	20	22	42	21	21	42
4	3	21	20	41	18	23	41
5	6	22	22	44	17	32	49
8	7	17	27	44	17	33	50
9	10	19	21	40	15	30	45
12	11	20	26	46	15	24	39
13	14	18	22	40	19	28	47
16	15	20	23	43	18	21	39
17	18	22	23	45	26	22	48
20	19	16	20	36	24	19	43
21	22	17	16	33	20	15	35
24	23	21	15	36	22	15	37
25	26	22	13	35	20	14	34
28	27	16	23	39	22	15	37
29	30	20	19	39	18	20	38
32	31	15	18	33	17	22	39
Mean		19	20·5	39·5	19	22	41

The differences have no statistical significance. The attack was evenly distributed over the plots so that relatively one plot did not suffer much more than another. Henceforward the attack subsided and new shoots sprang up presumably mostly tillers so that by the beginning of August the stand on all the plots appeared to be good and even.

*F represents mosaic-free plots and M mosaic plots.

No damage was done by cane diseases caused by fungi as there were no stem diseases and the leaves were remarkably free from spots and there was none caused by animals, the field being surrounded by a jackal and porcupine-proof fence. At harvest time the plots were examined under the direction of the Imperial Entomologist and Table III gives the percentage of insect infection. Not every cane in the whole experiment was examined but from six to twelve hundred canes in each plot, usually those in one or two rows.

TABLE III.

Percentage of insect infection at harvest.

F*	M*	F				M			
Plots		Top shoot borer	Stem borer	Root borer	Termites	Top shoot borer	Stem borer	Root borer	Termites
1	2	..	..	..	..	..	..	..	..
4	3	19·2	1·2	4·3	0	10·7	1·2	7·2	0
5	6	18·8	1·7	4·1	0	32·6	2·4	3·4	·04
8	7	21·1	2·0	4·3	3·1	26·6	1·3	2·5	0
9	10	20·1	1·8	5·0	1·7	18·0	1·8	4·0	1·9
12	11	13·3	1·7	5·3	1·5	18·8	3·0	4·8	4·8
13	14	11·5	2·3	3·2	1·9	14·9	2·6	4·7	1·8
16	15	10·6	1·2	4·2	1·1	15·3	2·0	6·7	1·9
17	18	16·2	2·6	4·2	1·5	15·7	1·8	4·6	·8
20	19	12·4	2·2	5·2	1·4	16·6	1·6	4·6	3·1
21	22	13·8	1·1	6·7	2·8	14·1	1·4	3·8	1·8
24	23	14·0	1·5	4·7	1·7	8·8	1·1	5·4	2·4
25	26	12·6	1·5	5·2	2·2	10·5	1·8	4·4	1·7
28	27	11·7	2·1	3·4	1·8	12·5	1·3	2·6	2·1
29	30	11·4	2·3	..	2·1	8·5	1·1	..	1·1
32	31	9·7	1·5	..	·8	11·2	1·6	..	1·4
Mean		14·4	1·7	4·6	1·5	15·7	1·7	4·5	1·6

The top-shoot borer is *Scirpophaga nivella* Fabr., the stem-borers are *Argyria sticticraspis* Hmpsn., *Diatraea venoseta* Swinh., and *Chilo zonellus* Swinh., and the root borer is *Emmalocera depressella* Swinh.

The mean difference in each case is small and has no statistical significance, so that whatever loss was caused by these insects was so evenly distributed that it did

*F represents mosaic-free plots and M mosaic-infected plots.

not have any effect on the difference of the mean tonnage of the plots. The bored shoots were not weighed separately as such a task was beyond the powers of the staff while harvesting.

The vicissitudes of this experiment show how nearly it was destroyed by the attack of insects. The plots were no worse off than other cane plots on the farm and indeed they compared favourably in appearance with the best on the farm and in the surrounding country. Insect attack on cane does an amount of damage in North Bihar far greater than is generally realised and the position calls for much greater effort in investigating the habits of these insects with a view to devising means of reducing the loss they cause which falls almost entirely on the grower.

Samples of cane were taken between the 22nd and 26th of February 1932 for analysis. The sample from each plot consisted of seven feet of cane taken at random from each of four lines, and the cane was crushed in the same bullock-driven, three-roller, iron mill as was used last year. The analysis of the juice was furnished by the Imperial Agricultural Chemist, and the details are as follows :—

TABLE IV.

Sugarcane tonnage plots 1931-32, mosaic-free versus mosaic, samples taken from 22nd-26th February 1932.

Plot Nos.		Weight of cane in lbs.		Weight of juice in lbs.		Percentage weight of juice to cane		Brix corrected		In juice					
										Sucrose per cent.		Glucose per cent.		Purity per cent.	
F* 1	M* 2	F 139	M 136·5	F 92·5	M 88·5	F 66·5	M 64·8	F 19·47	M 18·47	F 16·34	M 15·21	F ·63	M ·67	F 83·90	M 82·34
4	3	163	140	106·5	93	65·3	64·4	18·98	18·71	16·05	15·75	·59	·49	84·58	84·18
5	6	136	109·5	85·5	68·5	62·8	62·5	18·75	18·31	15·45	14·87	·77	·72	82·39	81·20
8	7	150	147	98·5	94·5	65·6	64·2	19·27	18·97	16·07	15·77	·70	·49	83·39	83·15
9	10	179	141	120	92	67	65·2	19·44	19·05	16·42	16·01	·59	·57	84·45	84·02
12	11	147·5	141·5	96·5	92	65·4	65	19·62	19·29	16·55	16·16	·50	·50	84·34	83·79
13	14	127·5	129·5	83·5	85	65·4	65·6	18·77	18·77	15·52	15·60	·65	·59	82·68	83·12
16	15	140	105	90	67·5	64·2	64·2	19·64	18·51	16·57	15·28	·55	·63	84·36	82·53
17	18	121	106	78·5	68	64·8	64·1	19·01	18·65	15·73	15·23	·66	·75	82·75	81·64
20	19	123·5	104	79·5	67	64·3	64·4	19·37	18·63	16·32	15·15	·66	·82	84·26	81·34
21	22	143	113·5	92·5	73	64·6	65·1	19·17	18·51	16·15	15·14	·50	·63	84·25	81·82
24	23	126·5	138·5	80·5	88	63·6	63·5	19·62	19·24	16·48	16·15	·53	·57	84·03	83·94
25	26	131·5	145	83·5	95	63·4	65·5	19·72	19·24	16·73	16·14	·51	·51	84·81	83·90
28	27	143	128	94·0	84	65·7	65·6	19·64	17·60	16·46	14·06	·58	·85	83·84	79·92
29	30	146	139	94·5	87	64·7	62·5	19·91	19·38	17·07	16·35	·47	·50	85·72	84·36
32	31	121·5	132	78	84·5	64·1	64·0	19·75	19·45	16·12	16·80	·50	·52	81·63	86·38
Mean . .		139·9	128·4	90·9	82·8	64·8	64·4	19·38	18·80	16·27	15·60	·587	·613	83·84	83·09
Difference .		—11·5		—8·1		—·4		—·58		—·67		—·026		—·75	

* F indicates mosaic-free plots and M mosaic-infected plots.

The hydrogen-ion concentration of the juice was taken immediately it was extracted from the cane, by the quinhydrone method using K-type potentiometer, and the values are given in Table V. The mean difference is within the limits of the error of experiment and so has no significance.

TABLE V.

Hydrogen-ion concentration of juice.

Plot Nos.	F*	1	4	5	8	9	12	13	16	17	20	21	24	25	28	29	32	Mean
	M*	2	3	6	7	10	11	14	15	18	19	22	23	26	27	30	31	
	F	5·85	5·78	5·85	5·80	5·83	5·72	5·82	5·82	5·87	5·81	5·87	5·81	5·75	5·81	5·78	5·85	5·81
	M	5·80	5·76	5·77	5·88	5·77	5·85	5·83	5·83	5·74	5·83	5·84	5·83	5·88	5·91	5·72	5·84	5·82

At the time of harvest between 22nd and 29th February 1932 three yards of cane from the ends of the rows and the extra plots at the two extreme sides were removed to eliminate edge effect. Then ten yards from the end of each plot were dealt with separately in order to get a length the same as in last year's experiment. As it worked out the length of 60 yards gave no better result than that of 50. Thus there were left, in each plot five rows of cane in an area five yards by fifty yards. The weights in maunds of stripped cane from the plot are as follows, one maund being equal to 82·28 pounds avoirdupois.

TABLE VI.

Weight in maunds of stripped cane.

F*	M	Mosaic-free	Mosaic
1	2	38·71	33·60
4	3	47·32	40·57
5	6	45·10	34·61
8	7	44·32	36·96
9	10	44·30	38·61
12	11	45·76	38·94
13	14	44·72	34·36
16	15	44·25	35·31
17	18	43·27	37·56
20	19	47·28	38·46
21	22	42·50	38·00
24	23	48·27	39·71
25	26	46·22	44·28
28	27	47·19	39·13
29	30	44·04	40·53
32	31	44·20	40·15
Mean		44·84	38·17
Difference		6·67 or 14·8 per cent.	

* F indicates mosaic-free plots and M mosaic-infected plots.

The statistical figures to determine the significance of the difference in yield between the series of pairs of plots are summarised below :—

Co. 213	Mean difference	Standard deviation	Student's method	
			Mean difference / Standard deviation	Odds
Cane	6·67	2·39	2·8	Very great
Juice	8	10·7	·75	170 : 1
Percentage juice to cane . . .	·4	1	·4	7 : 1
Brix	·58	·15	3·8	Very great
Sucrose	·67	·50	1·3	9999 : 1
Glucose	·026	·08	·32	6 : 1
Purity	·75	1·81	·41	13 : 1

Thus the differences of weight of cane, weight of juice, brix and sucrose are all statistically significant but not those of percentage of juice to cane, glucose and purity. This year then the loss in weight of stripped cane was 14·8 per cent., while the quality of the juice had deteriorated as shown by a small decrease in brix and sucrose. The loss in such conditions therefore would be about 15 per cent. of cane to the grower and 4 per cent. of sucrose to the manufacturer.

Examined during July 1931 Co. 213 cane on eight estates scattered fairly regularly along a line of 120 miles showed by count an average infection of ·2 per cent. with a range from 0 to 4 per cent., and this is still a low level of infection. So far the disease has caused only a small loss to the sugar industry in North Bihar but the analysis of this year's experiment shows how great that loss may become if mosaic disease were to spread to any great extent.

Summary.

In sixteen pairs of plots, each 5×50 yards, after removing cane to eliminate edge effect, mosaic-infected Co. 213 cane was 11 per cent. less in germination after seven weeks, 14·8 per cent. less in yield of stripped cane, 8·9 per cent. less in yield of juice, slightly less in brix and 4 per cent. less in sucrose than mosaic-free cane. A slight increase in glucose and decrease in percentage of juice to cane and in purity were not statistically significant.

STUDIES IN INDIAN PULSES.

A NOTE ON THE CYTOLOGY OF 'KABULI' AND 'DESI' GRAM TYPES

BY

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(With Plates XLI—XLIII).

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I. Introduction.

Attempts to obtain crosses between the white large seeded 'Kabuli' gram and the red or brown seeded 'Desi' gram have often met with failure. With great difficulty a few hybrid seeds were obtained from crosses between Pusa type 1 and Pusa type 25, and from crosses between type 2 and type 18. [Shaw, 1925] The setting percentage in these crosses was very low. The F_1 and subsequent generations were grown in the following years. Some very interesting results were obtained in this genetical study. Many new forms widely different from the parents made their appearance. The interpretation in F_2 and F_3 populations on the basis of segregation and interaction of factors proved a very complex problem in Mendelian inheritance.

The rapid progress in this genetical study of the crosses between 'Kabuli' gram and ordinary gram types awakened the realization that to understand more clearly the mechanism of heredity, a cytological knowledge of the material was essential. Differences found between the chromosome numbers of the giant mutant and gram type 22 from which the mutant has arisen [Dixit, 1931] gave a further impetus to carry on this investigation.

Therefore a cytological study of the parent types 1 and 2—the large white seeded ' Kabuli' types—and types 18 and 25 the ordinary gram types was undertaken. The results of this investigation together with a discussion as to the desirability of treating ' Kabuli ' gram as a distinct species are included in this paper.

My heartfelt thanks are due to Dr. F. J. F. Shaw, Imperial Economic Botanist, Pusa, for his guidance and to Mr. R. D. Bose, Special Research Assistant, for his constant help during the progress of the work.

II. Material and methods.

For studying the diploid number of chromosomes, seeds of Pusa types 1 and 2, the ' Kabuli ' gram types, and 18 and 25, the ' Desi ' gram types, were germinated. Their root-tips when about 1 cm. long after germination were cut and fixed between 12 and 1 o'clock in the noon in Allen's modification of Bouin's fluid. This fixative (picric acid, saturated solution in distilled water 75 parts, formalin 15 parts, glacial acetic acid 10 parts and urea 1 part) was found to be the most suitable for such studies; it penetrates rapidly fixing equally well at the centre and the periphery. The material was kept in the fixative over night and then washed and dehydrated in grades of alcohol rising from 20 per cent. by 5 to 90 per cent. and absolute. Clearing was done in grades of xylol and the material was embedded in 52°—56° paraffin. Sections 10 to 12 μ thick were cut and stained according to Haidenbain's iron-alum-hæmatoxylin methed. Chromosome counts were made in the metaphase stage in all the types. To confirm the results of the counts made in the somatic cells, it was considered advisable to find out the haploid number of chromosomes as well. For this purpose smears of pollen mother cells according to modified smear method [Dixit, 1931] were made. But the preparations were stained in Delafields Hæmatoxylin according to Milton's Schedule [1929] and the results obtained were better than the previous.

III. Diploid number of chromosomes.

The only work on the cytology of *Cicer arietinum* L. to which I have found any reference in literature is that of Dombrowsky-sludsky [1927]. He studied the somatic chromosomes and stated that the number and form of chromosomes in *Cicer arietinum* L. is the same as in *Pisum sativum* (14). They are smaller in the former and one pair of chromosomes has acolytes (satellites). Rau [1929] has also made a reference to the somatic chromosome number in *Cicer arietinum*. He, confirming the results obtained by Dombrowsky-sludsky, states that a remarkable feature in the rapid and enormous increase in the size of the nucleolus in early prophase stages, with a subsequent reduction in the size as the chromosomes complete their growth—indicating that the nucleolus prepares or provides material

for the growth of chromosomes. Rau has made no mention as to the variety of gram in which he made the chromosome counts—whether it was 'Kabuli' or 'Desi' type of gram—and no illustrations are given. The present writer has counted the chromosomes in somatic cells of the root tips in Pusa types 1, 2, 18 and 25 and found that in types 18 and 25, which are the commonly cultivated types of 'Desi' gram, the diploid number of chromosomes is 14 (Plate XLI, figs. 1 and 2) as stated by Dombrowsky-sludsky [1927] and confirmed by Rau [1929]. But in types 1 and 2 the 'Kabuli' gram types the diploid number of chromosomes was found to be sixteen (Plate XLI, figs. 3 and 4), instead of fourteen as reported for the ordinary gram types. The presence of eight pairs of somatic chromosomes in 'Kabuli' types 1 and 2 is quite clear from figures 3 and 4 as observed in the metaphase stage. It has not been possible to find out which particular extra pair of chromosomes is present in 'Kabuli' gram and absent in the 'Desi' gram. There appear to be 3 pairs of V-shaped chromosomes in both the 'Kabuli' and 'Desi' gram types, the rest of them being somewhat rod-shaped. One pair of rod-shaped chromosomes is definitely longer than the others in all the four types.

IV. Haploid number of chromosomes.

No reference could be found in the literature about the haploid number of chromosomes in *Cicer arietinum* or any other species of the genus cicer. In the list published by Gaiser [1930] somatic number of chromosomes in *Cicer arietinum* is given, but no mention is made about the haploid number. The omission is perhaps due to the difficulty which is experienced in fixing the floral buds in the proper stage. The writer studied the haploid number by making smear preparations of the pollen mother cells. It was found that in types 18 and 25, the 'Desi' gram types, the n-chromosome number was seven (Plate XLII, figs. 5 to 8 and 9 to 12) which was completely in accordance with the observations made in the somatic cells. In types 1 and 2, the 'Kabuli' gram types, it was observed that the n-chromosome number was eight (Plate XLIII, figs. 13 to 17 and 18 to 22). This confirmed the results obtained from the study of somatic cells and it was established that the number of chromosomes in 'Kabuli' gram was greater than in the 'Desi' gram types. The counts were made at the first as well as the second division of the pollen mother cells. A few stages showing the reduction division have been given in Plates XLII and XLIII, but no effort has been made to study the Miosis in detail.

V. Discussion.

The existence of remarkable differences between the 'Kabuli' and the ordinary cultivated varieties of gram has long been recognised by various workers—plant

breeders as well as the taxonomists. Howard, Howard and Khan [1915] state that the large ' Kabuli ' gram types stand out quite clearly from the rest of the crop as cultivated under field conditions in India. They have a light foliage, white or whitish green flowers and are considerably taller with larger leaves and leaflets than the country crop. Their seeds are much larger than that of ordinary gram and are always whitish in colour. Watt [1908] has gone so far as to state that besides the common cultivated Indian gram types there is a very special variety, or perhaps a distinct species known as ' Kabuli ' gram, which has experimentally been grown in India for a century or more. He remarks that ' Kabuli ' gram plants are much more robust than the ordinary gram and have large white seed. It is apparently a form, peculiar to the country indicated by its name and has perhaps been imported into India very recently. Its poor distribution and scanty cultivation in India strongly support this view. From the first developmental stages to the last, ' Kabuli ' gram shows remarkable differences from the ordinary gram types. The seed germination tests that have been carried on from time to time at Pusa, show that the ' Kabuli' gram types take 3 to 4 days to put forth the radicle, while the ordinary gram types begin to germinate within 1 to 2 days. The ' Kabuli ' gram types are decidedly late in maturity as compared with other types and have a longer growing period. Percentage of sterile pods is also greater in these forms and they are comparatively low yielders.

The abovementioned facts, when put together with the remarkably big size of the plants, leaves, leaflets, flowers, pods and seeds and the constant whitish colour of the flowers and seeds in ' Kabuli ' gram, form a strong basis for treating it as a species quite distinct from *Cicer arietinum* L.

The difficulty with which crosses can be made between the ' Kabuli ' and ordinary gram types and the low fertility in some of their hybrids, fulfils another criterion for placing ' Kabuli ' gram in a separate species.

The result of the present cytological study of the ' Kabuli ' gram and ' Desi ' gram afford another weighty argument in favour of the above views. The diploid number of chromosomes is sixteen in the ' Kabuli ' gram instead of fourteen as reported for *Cicer arietinum* L. [Dombrowsky, 1927] and similarly the haploid number in ' Kabuli ' gram is eight and not seven as found out in the case of ordinary gram. The importance of chromosome numbers in taxonomic studies cannot be underestimated. They are of great value in investigations on natural grouping and the relationship of species within a genus, and have been utilised with advantage by Heilborn [1922] in his studies on the species formation and phylogany in the genus Carex, by Babcock and Navashin [1930] in their account of the genus Crepis. Compared with other morphological characters chromosomes have the advantage that they can be more objectively judged. They are further

more of special importance in species studies, as they are the carriers of hereditary characters.

It can not be definitely said whether the 'Kabuli' gram types grown in India have sprung up from a stock originally cultivated or wild in India or have been imported from the country indicated by their name. But from the study of the Gigas mutation in gram type 22 [Dixit, 1932] one thing appears very probable that the 'Kabuli' gram with large white flowers and seeds might have originated as a chromosomal mutation from some small white grained variety of gram. The 'Kabuli' gram like the Gigas mutant has large plants, with large leaves, leaflets, flowers, pods and seeds. The chromosome number is also similar in both, the diploid number being sixteen and haploid being eight. Thus the idea, that large white seeded 'Kabuli' gram with 16 chromosomes might have originated as chromosomal mutation from a small white seeded gram type with 14 chromosomes, is not far fetched. This case is very similar to the observed case of Gigas mutant arising from gram type 22.

The cytological evidence when put together with the remarkable differences in size between 'Kabuli' and 'Desi' gram types, seem to point to the conclusion that 'Kabuli' gram may be treated as a distinct species from *Cicer arietinum* L. It may be called as *Cicer Kabulium* and placed as a separate species in the section Arietaria of the genus Cicer (Engler and Prante.).

VI. Summary and conclusion.

1. The diploid number of chromosomes in 'Kabuli' gram is sixteen, instead of fourteen, as observed in the ordinary cultivated forms of *Cicer arietinum* L.

2. The haploid number of chromosomes is similarly 8 in 'Kabuli' gram and seven in ordinary types of *Cicer arietinum*.

3. A mutational origin of the white large seeded 'Kabuli' gram from white small seeded varieties of gram is indicated.

4. The cytological evidence coupled with the genetical evidence and differences in taxonomic characters point to the conclusion that 'Kabuli' gram be treated as a distinct species from *Cicer arietinum* L.

5. 'Kabuli' gram types may be placed in the species to be named *Cicer Kabulium*—in the section Arietaria of the genus Cicer and distinguished from other species of the group by its large white flowers and large white seeds, having 16 diploid and 8 haploid chromosomes.

VII. References.

Babcock, E. B. and Navashin, M. (1930) Bibliographica Genetica, Vol. VI, p. 2-90.

Dixit, P. D. (1931, 1). Report of the Imperial Economic, 1929-30, p. 25-26.

—— (1931,2). *Ind. J. Agric. Sci.*, **1**, part IV, p. 419-433.; II, Part IV, p. 491—407.

Dombrowsky-sludsky, L. (1927). *J. Soc. Bot. Russie* **12**, p. 162-72, Abst. Bot. Centrbl. N. F. **13**, p. 66.

Gaiser, L. O. (1930). Bibliographica Genetica, Vol. VI, p. 171-466.
Heilborn, Otto. (1922). *Hereditas* **9**, p. 245-246.
Howard, A., Howard, G. L : C. and Khan, A. R. (1915). *Mem. Dept. Agric. Ind., Bot. Ser.* 7. No. 6 p. 225-233.
Milton, J. W. (1929) Univ. Calf., Publ., Vol. 14, p. 345—362.
Rau, N. S. (1929). *J. Ind. Bot. Soc.* **8**, No. 3, p. 201-206.
Shaw, F. J. F. (1926). Report of the Imperial Economic Botanist, 1925-26, p. 22.
Watt, G. (1908). The Commercial products of India, p. 296.
Webbs, J. M. (1929). Univ. California, Publ. Botany, Vol. 14, No. 15, p. 345-352.

VIII. Explanation of Plates.

All the figures in plates XLI, XLII and XLIII are based on preparations of material in Allen's modification of Bouin's fluid and stained in iron-alum-hæmatoxylin in plates XLI and in Delifields hæmatoxylin in plates XLII and XLIII. These figures have been drawn with the aid of a Lietz Camera lucida. A Zeiss 1-12 inch homogene immersion objective has been used with K 12 Zeiss ocular, thus giving 1500 times magnification. But as the drawings have been made at the level of the base of the microscope and not at the level of the microscope stage, the magnification is proportionately greater in all cases. Wherever objective other than K 12 Zeiss has been used, mention has been made in individual explanation of figures.

Plate XLI. Somatic or diploid number of chromosomes in meta phase stage in 'Kabuli' and 'Desi' types of gram.

Fig. 1. 14-chromosomes in type 18 ordinary gram.
Fig. 2. 14-cromosomes in type 25 ordinary gram.
Fig. 3. 16-chromosomes in type 1 'Kabuli' gram.
Fig. 4. 16-chromosomes in type 2 'Kabuli' gram.

Plate XLII. Reduced or haploid number of chromosomes in 'Desi' gram types—with some division stage.

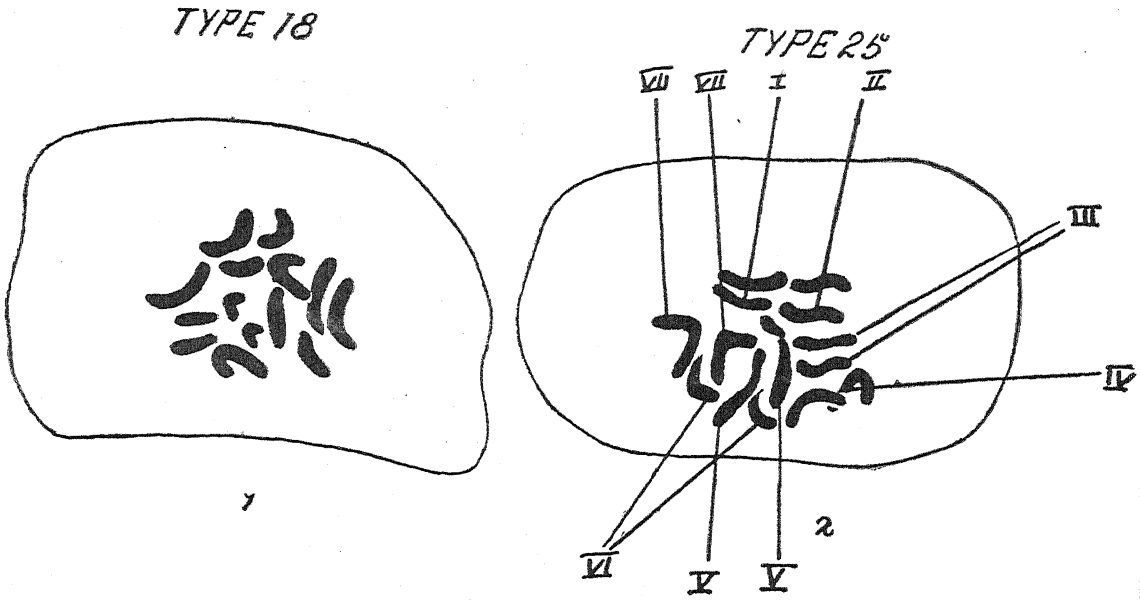
Fig. 5. 7-chromosome in metaphase stage type 18.
Fig. 6. Second division beginning of anaphase stage type 18.
Fig. 7. Seven chromosomes in late anaphase type 18 on either pole after heterotypic division.
Fig. 8. Late anaphase and early telophase in homoetypic division type 18.
Fig. 9. Metaphase 7 chromosomes just separating type 25.
Fig. 10. Metaphase 7 chromosomes side view type 25.
Fig 11. Beginning of homoetypic division. Note 3 long and 4 short chromosome pairs at one end type 25.
Fig. 12. Late anaphase and early telophase of homoetypic division type 25.

Plate XLIII. Reduced or haploid number of chromosomes in 'Kabuli' gram types with some division stages.

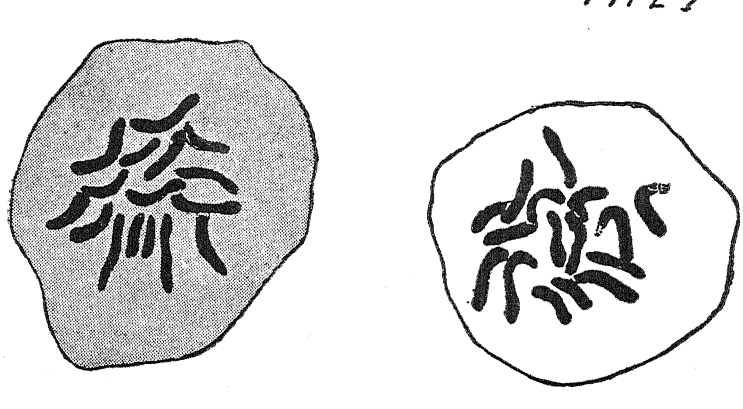
Fig. 13. Beginning of anaphase heterotypic division type 1.
Fig. 14. Side view metaphase stage of chromosomes type 1.
Fig. 15. Advance anaphase of heterotypic division type 1.
Fig. 16. } Anaphase of homoetypic division type 1.
Fig. 17. }
Fig. 18. Beginning of anaphase heterotypic division note 8 chromosomes type 2.
Fig. 19. Advanced anaphase heterotypic division type 2.
Fig. 20. Beginning of anaphase of homœtypic division type 2.
Fig, 21. Note 4 long and 4 shorter chromosomes type 2.
Fig. 20. Homoetypic anaphase advanced type 2.

DIPLOID NUMBER OF CHROMOSOMES

Desi GRAM.



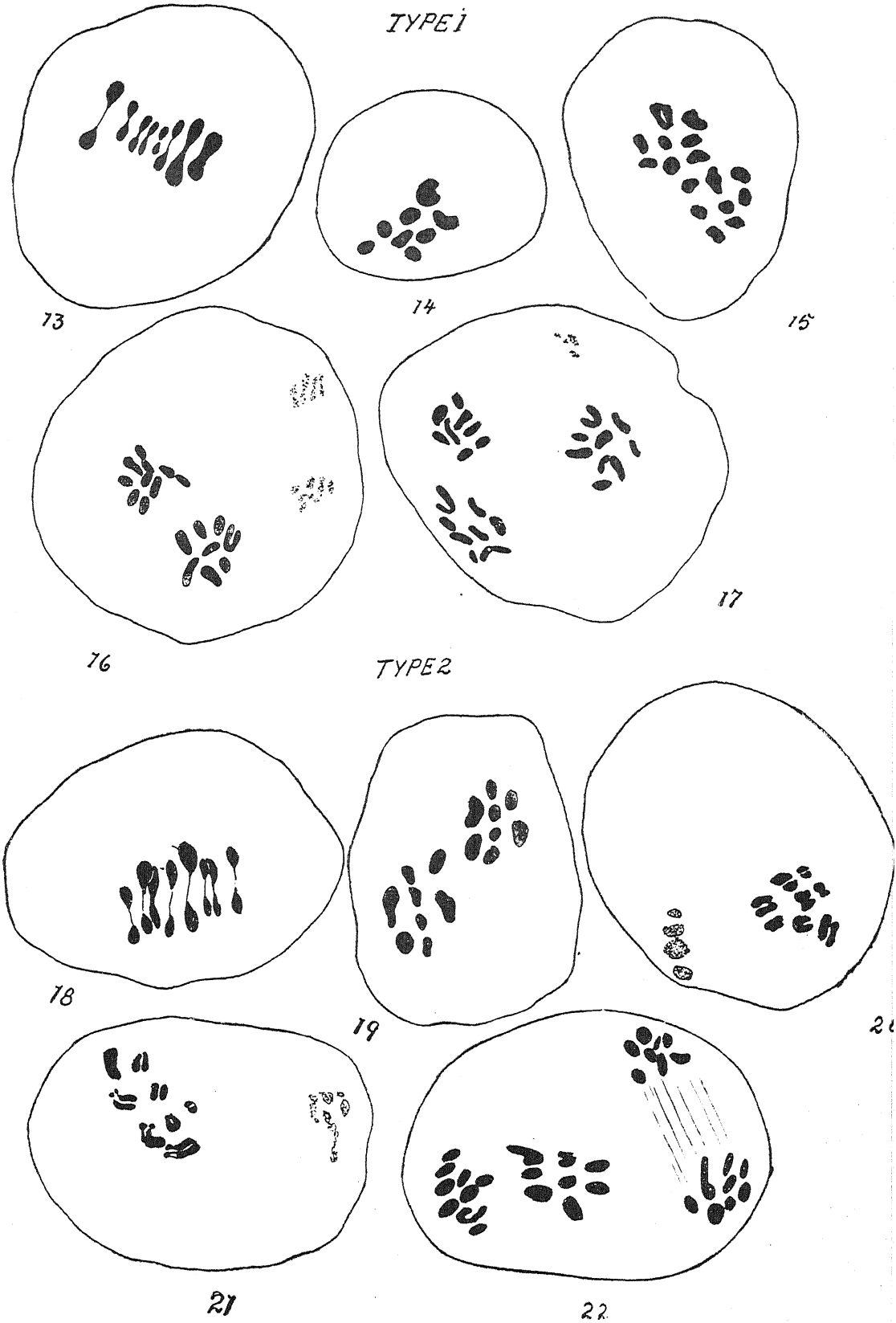
Kabuli GRAM.



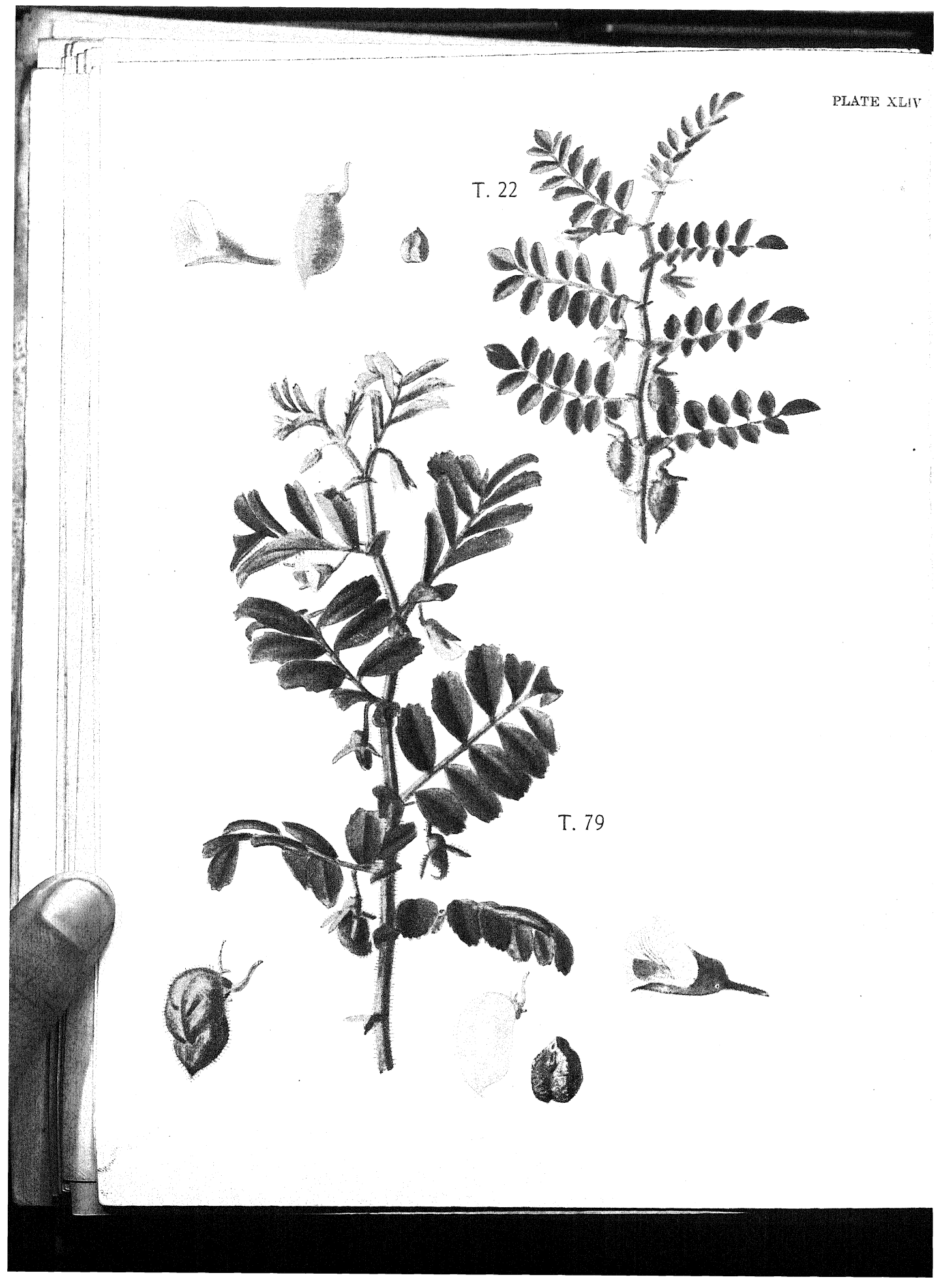
(*For explanation please see page* 390.)

HAPLOID NUMBER OF CHROMOSOMES

Kabuli GRAM.



(For explanation please see page 200.)


T. 22
T. 79

STUDIES IN INDIAN PULSES:

A CASE OF GIGANTISM IN GRAM (*CICER ARIETINUM*)

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I. INTRODUCTION.

The universality of the phenomenon of gigantism in plants is apparent from the pages of nurserymen's catalogues. The fact that seedsmen are able to offer pure seed of giant races, year after year, is sufficient evidence that gigantism is a 'fixed' character and that giants when self-fertilised breed true to type. The real nature of this phenomenon, however, is not clearly understood.

In many cases like that of (*i*) *Oenothera gigas* [Gates, 1909]; (*ii*) Some giant forms of *Primula sinensis* [Gregory, 1914 ; Somme, 1930]; (*iii*) Telham beauty the gigas form of *Campanula percicifolia* [Gairdner, 1926]; (*iv*) Giant varieties of

narcissus and hycanthesis [Mol De, 1922] and (*v*) New world cottons [Denham, 1924], the gigas nature of the plant is attributed to the doubling of chromosomes. This, however, is not always true. All cases of gigantism are not associated with tetraploidy. Gregory [1909] and Keible [1912] investigated the cytology of the gigas and ordinary forms of *Primula sinensis* and came to the conclusion that there is no increase in the number of chromosomes in the giant, but only an increase in their size. Similar results were obtained by Tischler [1918] in his studies on Phragmites communis, Stompes [1919] from his extensive studies of the semi-gigas and gigas forms of narcissus believes that as gigas forms are known to have occurred with and without doubling of chromosomes in narcissus and many other plants like primula, so such doubling of chromosomes is not the cause of giant mutations, but a character of the mutant.

Sinoto [1925] studied another interesting case of gigantism in Plantago. He states that *P. japonica* is a giant form conspicuously larger than P. major var asiatica. But the number of chromosomes in the giant is only half that of the ordinary P. major. The volume of the nucleus is less in the giant, but the cell size is about the same in both species. The only difference in the internal structure is that the cells are more numerous in the giant than the ordinary form.

The mutation in *Saccharum spontaneum* affords another example of gigantism [Bremen, 1928]. Here the chromosome number in the nine giant plants studied varied between 48—56 instead of being 40 as is the case in the parent variety.

From the abovementioned cases of gigantism it is evident that the giant form may differ from the parent form in a variety of ways. To understand this phenomenon more clearly it is essential to have a thorough knowledge of the environmental factors under which the giants arise and also to carry on extensive comparative studies of the morphology, histology, cytology and genetics of the giant form and the parent type from which the giant is supposed to have arisen.

In the present paper the author has given some observations on the comparative morphology and cytology of a giant mutant which first made its appearance in gram type 22 in the year 1927-28 [Shaw, 1929]. The role of mutation factor in the origin of new species in the genus cicer has also briefly been discussed in the following pages.

I am very much indebted to Dr. F. J. F. Shaw, Imperial Economic Botanist, for his guidance and Mr. R. D. Bose, Special Research Assistant, for his constant help during the progress of the work.

II. Origin of the giant mutant in gram type 22.

Before giving an account of the origin of the giant mutant it will be worth while to give a brief history of the parent type from which the mutation has origi-



Gigas Mutation.



Type 22.

nated. Gram type 22 was isolated by Howard, Howard and Khan [1915] from a seed sample received from Aligarh (U. P.) in the year 1909. They classified it in the second group, *with plants, leaves, flowers and seeds small* and described it as follows :—

> " Type 22. Early, habit erect, leaves light bluish green. Flowers pink ; standard light pink, wings slightly bluish. Seeds small dark brown."

This type was propagated since then from single plant seed as a pure line and was breeding true to type. In the year 1927-28 [report * of the Imperial Economic Botanist, year 1929] suddenly three plants appeared in this culture which were distinctly different from type 22. It appeared to be a case of gigantism as the plants and all their organs, leaves, flowers, pods and seeds showed an enormous increase over the type. Unfortunately only one of these three plants gave fertile seed.

These seeds were sown in the following year (1928-29), whole of the progeny bred true to the gigas character. At the same time two plants of the giant mutation type again appeared in the parent culture, but formed no seed. One plant of the gigas kind was observed in the year 1929-30 as well. In 1930-31 a large population of type 22 was grown from single plant seed. The seeds were examined before sowing to make sure that there was no admixture. But out of this population of over a 1,000, five plants of the mutant type were observed. These could be spotted out about a month after germination. From this it is apparent that type 22 is in a mutable stage. The mutations that have occurred in this type from year to year, unlike the mutations in oenothera and datura, are all alike and resemble in external form the giant mutants which appeared first in 1927-28. The giant mutant of 1927-28 has been propagated since then and is breeding true, no intermediate form or forms resembling the parent type have been noticed even in populations of many hundred plants of the gigas culture.

III. Morphology.

There is an enormous increase in the size of the giant mutation plants and all their organs over the parent type 22. In the following pages a comparative account of the morphological characters is given.

(a) *Root system—Plate XLV.*

The root systems of the giant mutant and the parent type 22 were examined when the plants had begun to flower. The roots were uncovered with a stream of water from a knap sack sprayer.

* In the report 1927-28 through oversight type 24 was published as the parent stock instead of type 22 from which the gigas mutation arose.

Type 22.—The tap root is prominent and goes vertically downwards reaching a maximum depth of 60 cms. Its diameter near the soil surface is about 7 to 8 mm. It tapers downwards and bears laterals up to 14 cms. of its length. These laterals are 2 to 1 mm. in diameter and 30 to 50 cms. in length. They are branched and rebranched in turn forming a good fibrous absorbing system in the first 28 to 30 cms. of the soil surface. The main tap root bears only very few branches after 14 cms. or so of its length.

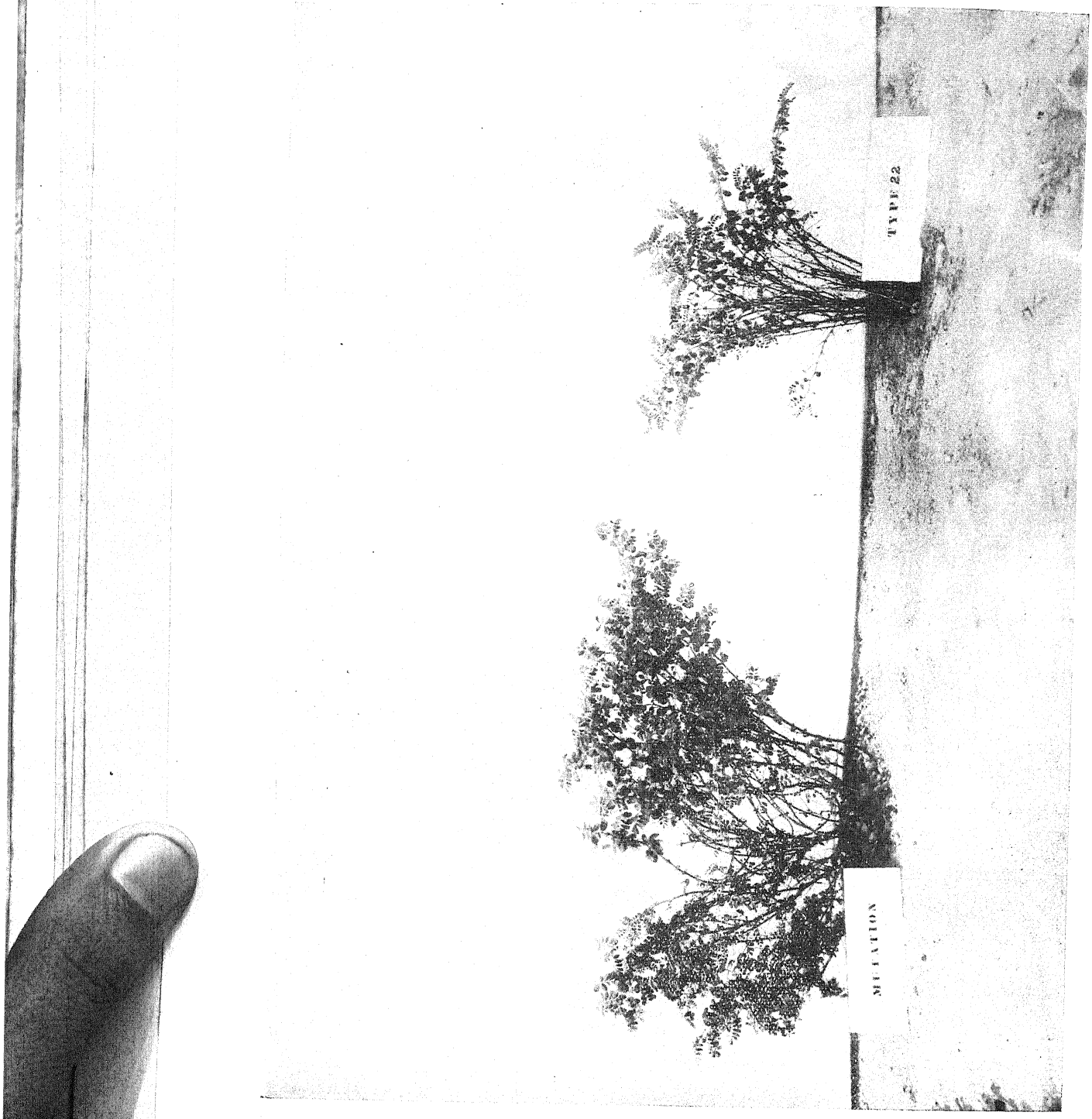
Gigas mutant.—The tap root is thicker than type 22, diameter near the surface being 10 to 12 mm. It tapers downwards as in the previous case, but the difference in thickness is maintained throughout. It reaches a maximum depth of 66 cms. It bears thick laterals up to 17 to 18 cms. of its length. The number of laterals is also greater. The difference in the thickness of the laterals is well marked, their diameter sometimes being up to 3·5 mm. The length of the laterals is not significantly greater than in the parent type, but they are more profusely branched and form a very dense absorbing root system in the upper 30 to 34 cms. of the soil surface. The root nodules are more abundant and bigger in size as compared with the parent type.

The root system on the whole in the gigas mutant is more vigorous than the type. The main root as well as the laterals are distinctly thicker and more profusely branched. They are longer also, but the increase in length is not very great.

(*b*) *Stem.*

The main stem like the tap root is thicker in the gigas mutant in comparison with the parent type, and so are the branches. The average diameter of the main stem near the base being 14·5 mm. in the former and 9·3 in the latter. The branches are more numerous in the gigas form than in the parent type. Measurements of the length of the longest branch in 100 different mature plants of each of the gigas and type 22 are given in Table I. The average mean length of the branch in the gigas is 56·97 cms. and in the parent type 53·0 cms. The difference being only 4 cms. It will be noticed that there is not so much increase seen in the length of the stem and the root as is in their thickness in the mature plant. It seems probable that the further growth in height of the mutant is checked due to the setting in of unfavourable hot winds long before it has attained its full height. Observations taken in early life of the plants to compare the length of the branches in the type and the mutant show greater differences than those which are recorded when the plants are mature. It can safely be asserted that increase in length of the branches in the mutant over the type would have been greater than is seen in Table I if the growing season had been longer in this part of the country.

PLATE XLVI



It is very remarkable that the distance between the insertion of the successive leaves on the branches is much longer in the gigas than the parent type, *i.e.*, the internode length in the type is much smaller as compared to that of the gigas form. This is responsible for the fewer number of leaves on the branches in the mutant and greater number of leaves in the type. There are about 35 leaves on the average on the main branch in the parent type, while in the gigas mutant their number is only 24.

TABLE I.

Main branch length and number of leaves.

Length of the main branch cm.	Frequencies in		Number of leaves on the main branch	Frequencies in	
	Parent type 22	Gigas mutant		Parent type 22	Gigas mutant
46—48	5	1	14—17	...	2
49—51	28	7	18—21	...	24
52—54	35	27	22—25	1	41
55—57	26	36	26—29	15	25
58—60	6	18	30—33	16	8
61—63	...	8	34—37	30	...
64—66	...	3	38—41	27	...
			42—45	9	...
			46—49	2	...
Total .	100	100	Total .	100	100
Mean .	53·0	56·97	Mean .	35·58	23·9

(*c*) *Leaves.*

The leaves of the gigas form are remarkably different from the parent type in their colour, shape and size and occur at greater distance from one another in the former as compared with the latter. They are characterised by the enormous size and peculiar shape of their stipules and leaflets.

Colour.—The general colour of the leaves has been described [Shaw and Khan, 1931] as light bluish green for type 22 and light green for the mutant. A slightly yellowish tinge in the gigas mutant makes its foliage look a bit lighter than the parent type. When individual plants are compared under field conditions gigas

mutant stands out quite clearly from all the other 83 types in foliage colour. There is a marked red colouration on the entire margin of the leaflets and the stipules, in the gigas form, which is entirely absent in the type. Red colouration is also present in the leaf axils of the mutant.

Shape.—The leaves are bipinnate in both the forms. But the shape of the leaflets in the gigas form is peculiar from all the other gram types. The leaflets are broader towards the upper side and become narrow downwards, thus their maximum breadth is not in the middle, as in the leaflets of other types. The dentation is confined to the upper one half of the leaflet in the gigas form, while it extends to about two-thirds the margin in the ordinary type. The tip of the leaflet in the mutant is not pointed as in the parent type (Plate XLIV).

Size.—Table II gives a record of measurement of two hundred leaves each of the mutant and the parent type taken from 100 different plants. Each leaf represents the largest leaf on the main stem present at the time of picking. The distance from the base of the leaf to the tip of the terminal leaflet has been taken as the length of the leaf. The length of the leaf varies from 73 mm. to 99 mm. in the gigas mutant, the mean being 83·24 mm. and in the type it varies between 49 and 72 mm. the mean being 59·29 mm. only. Thus the leaf length in the gigas form is about 24 mm. greater than in the type, *i.e.*, the mutation plant leaf is about one and a half times longer than the leaf of the parent type.

A comparison of the size of the leaflet in the two forms (shown in Table II) reveals that the leaflet of the gigas is twice as big as that of the parent form. The average mean length being 13·08 mm. and breadth 6·62 mm. in the type while in the gigas the length and breadth is respectively 24·9 mm. and 14·5 mm.

TABLE II.

Leaf and leaflet measurements.

Leaf length mm.	Frequencies in		Leaflet length mm.	Frequencies in		Leaflet breadth	Frequencies in	
	Parent type 22	Gigas mutant		Parent type 22	Gigas mutant		Parent type 22	Gigas mutant
49—51	4	...	9—10	8	...	4—5	9	...
52—54	16	...	10—11	15	...	5—6	50	...
55—57	53	...	11—12	23	...	6—7	68	...
58—60	65	...	12—13	38	...	7—8	59	...
61—63	45	...	13—14	56	...	8—9	10	...

TABLE II—*contd.*

Leaf and leaflet measurements—contd.

Leaf length mm.	Frequencies in		Leaflet length mm.	Frequencies in		Leaflet breadth	Frequencies in	
	Parent type 22	Gigas mutant		Parent type 22	Gigas mutant		Parent type 22	Gigas mutant
64—66	14	...	14—15	44	...	9—10	4	...
67—69	2	...	15—16	14	...	10—11	...	...
70—72	1	...	16—17	2	...	11—12	...	5
73—75	...	8	20—21	...	5	12—13	...	25
76—78	...	19	21—22	...	13	13—14	...	46
79—81	...	48	22—23	...	17	14—15	...	51
82—84	...	51	23—24	...	24	15—16	...	47
85—86	...	42	24—25	...	31	16—17	...	15
88—90	...	18	25—26	...	48	17—18	...	9
91—93	...	9	26—27	...	32	18—19	...	1
94—96	...	4	27—28	...	21	19—20	...	1
97—99	...	1	28—29	...	5			
			29—30		4			
Total	200	200	Total	200	200	Total	200	200
Mean	59·29	83·24	Mean	13·08	24·9	Mean	6·62	14·5

Stipules.—There is no numerical data as regards the size of the stipules, but a glance at leaves in Plate XLIV will bring home to one's mind the enormous size of stipules in the gigas form as compared with type 22. The stipules in the gigas form are the biggest being three or four times in size of that of any other culture.

Number of leaflets.—In both the gigas and the parent form there are predominantly 6 pairs of leaflets. Table III shows the variation in this character. It will

be clear from this table that the proportion of leaves with smaller number of leaflets is greater in the gigas than the parent type.

TABLE III.

Number of leaflets per leaf in the gigas mutant and type 22.

Type	No. of leaves with 11 leaflets	No. of leaves with 12 leaflets	No. of leaves with 13 leaflets	No. of leaves with 14 leaflets	Total
Parent type	3	140	48	9	200
22	1·5 per cent.	70 per cent.	24 per cent.	4·5 per cent.	
Gigas mutant	26	166	6	2	200
79	13 per cent.	83 per cent.	3 per cent.	1 per cent.	

(*d*) *Flowers.*

Flowers also like all other organs are larger in the gigas mutant than the parent type. They have been described [Shaw and Khan, 1931] as small, solitary, pink, standard and wings pale pink with a general bluish tinge in type 22, while in the mutant they are large, solitary pink, standard and wings pink with a deep bluish tinge, *i.e.*, the colour intensity in the flowers of the gigas form is deeper than in the parent type and blue colouration in the wings is more prominent.

Measurements of the length and breadth of the standard and the length of the calyx tube have been tabulated in (Table IV). These are the averages of 100 flowers picked fresh from different plants of the gigas mutant and the type.

TABLE IV.

Flower measurements.

Type	Average length of the standard	Average breadth of the standard	Average calyx length
Parent type 22	9·25	8·5	6·95
Gigas mutant	10·9	12·36	10·63

It will be seen from the above table that the breadth of the standard in the gigas is nearly one and a half times that of the type, while the length of the standard in the former is only about 1·5 mm. more. Another fact to be noticed is that the length of the standard is greater than the breadth in the parent type, but in the gigas form the case is just the reverse; the breadth of the standard being greater than its length.

The length of the calyx measured from the base of the calyx tube to the tip of the calyx tube is on the average 6·95 in the type and 10·63 in the gigas mutant, *i.e.*, the calyx is more than one and a half times longer than the parent type.

(*e*) *Pods.*

The characteristic shape (Plate XLIV) and abnormally big size of the pods is a distinguishing feature of the gigas mutant. Grooves on the surface is one of their interesting features. The colour of pods goes on changing with maturity, but throughout gigas pods appear to be lighter in shade than the pods in the parent type. Table V gives the average length and breadth of the pods and length of the pedicel in the gigas form and the type.

TABLE V.

Pod measurement.

Type	Length of the pod mm.	Breadth of the pod mm.	Length of pedicel
Parent type 22	22·05	13·1	10·3
Gigas mutant	39·3	25·78	11·6

It will be seen from the above table that length as well as breadth is nearly twice as much in the gigas pods as in the type; the pedicel is also about 1·3 mm. longer in the gigas form.

(*f*) *Seeds.*

The seeds of the gigas mutant are not only bigger in size than the parent type, but they also differ in shape and colour (Plate XLIV). In the type the seeds are dark reddish brown in colour and have a smooth surface, while in the mutant the seeds are darker in shade and have a rough granulated surface. The seeds in the gigas form are characterised by the presence of deep grooves on the sides and on the convex dorsal surface which are absent in the type. The weight of 1,000

grains of the mutant and the type and their volume as stated below gives a fair idea of the size of gigas seeds.

TABLE VI.

Type	Weight of 1,000 grains	Volume of 1,000 grains
Parent type 22	120 gr.	101 c. c.
Gigas mutant	225 gr.	198 c. c.

The seeds like the pods in the mutant are about twice in size as compared with the parent type.

This extraordinarily big size of the gigas seeds accompanied by the deep grooves on their sides and the dorsal surface will form one of the distinguishing features of the gigas species.

(*g*) *Sterility.*

The gigas mutation when it first appeared in the year 1926-27 in type 22 seemed to be highly self-sterile. Out of three giant plants, that appeared, only one set a few seeds. But its propagation in successive years has not shown so much sterility. In the year 1928-29, 500 mature pods from 5 or 6 different plants of the gigas and type 22 were examined and it was seen that there were about 21 per cent. sterile pods in the gigas mutant and 18 per cent. sterile pods in type 22. The results are tabulated below and they show the percentage of one seeded—two or more seeded and of sterile or no-seeded pods. In the gigas form the pods are predominantly one-seeded, while in the type there is an equal percentage of two-seeded and one-seeded pods.

TABLE VII.

Percentage of one seeded, two or more seeded and sterile pods in gigas mutant and type 22 in 1928-29.

Type	1-seeded pods	2 or more seeded pods	Sterile pods
Parent type 22	41 per cent.	41 per cent.	18 per cent.
Gigas mutant	73 per cent.	6 per cent.	21 per cent.

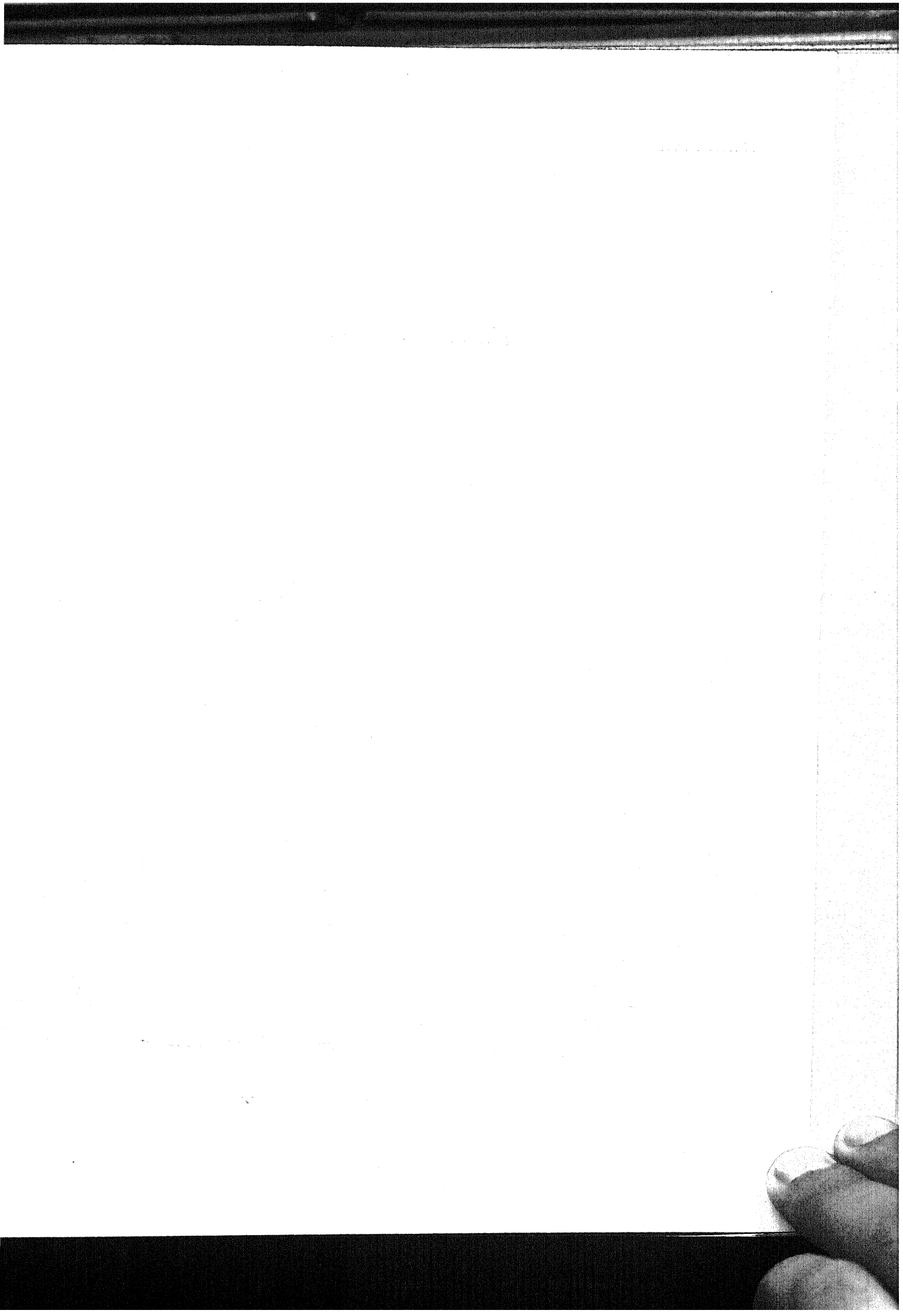
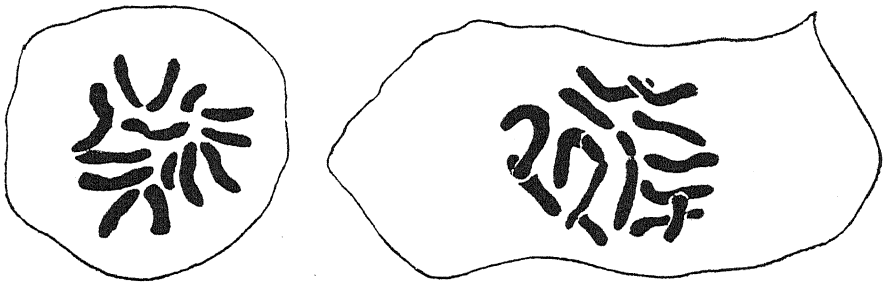


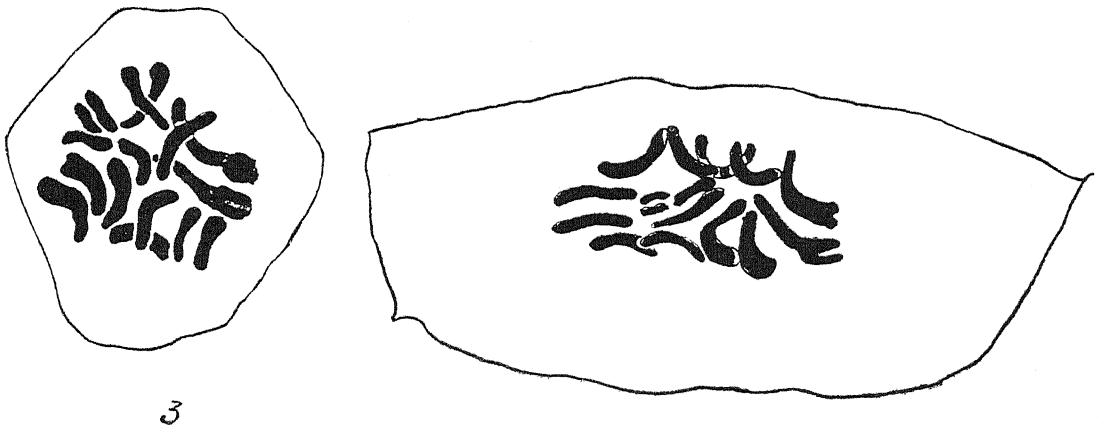
PLATE XLVII

GRAM TYPE 22



1 2

GIGAS MUTATION



3 4

(k) *Flowering and maturity.*

Parent type 22 is an early type. It begins to flower at Pusa by 10th to 20th of January, some 65-75 days after sowing, and is in full flower by the end of January or beginning of February. It is ready for harvest by the end of March. But the gigas mutation is one of the very late types. It begins to flower 15 to 25 days after the parent type and is in full flower only by the end of February or beginning of March. It is practically last to mature among the gram cultures and is not ready for harvest earlier than the end of April.

Thus this gigas mutation is remarkably later than the parent type in time of flowering as well as in maturity. This late flowering and maturity are characteristic of most of the giant mutants which have been recorded in oenothera, tobacco, primula and cotton, etc., and is generally attributed to slow rate of growth or greater vegetative development. But the problem is a very complicated one and requires careful investigation into the physical and physiological causes which are responsible for delayed flowering and maturity in the giant mutants. A comparison of the rate of transpiration, carbon assimilation and root absorption, accompanied by the rate of growth in the giant and the ordinary type will be of interest.

IV. Cytology.

(a) *Diploid number of chromosomes.*

Material and methods.—The material for studying the diploid number of chromosomes consisted of the root tips, obtained from germinating single plant seeds of the gigas mutant and the parent type 22. Root tips when ·5 to 1 cm. in length were cut and fixed in Allen's modification of Bouin's fluid (picric acid, saturated solution in distilled water 75 parts, formalin 15 parts, acetic acid 10 parts and a few crystals of urea). The root tips were kept over night in the fixative and then washed and dehydrated in the usual way in grades of alcohol. Xylol was used as the clearing agent and the material was embedded in paraffin. Sections 10-12μ thick were cut and stained in iron-alum-hæmotoxylin.

Chromosome counts.—For counting the number of chromosomes in somatic cells the metaphase stage is the most suitable, as all the chromosomes lie nearly in a single plane. In this case counts were made in longitudinal as well as transverse sections of the cells. Figures 1 and 2 (Plate XLVII) show the chromosomes in metaphase stage in type 22, while figures 3 and 4 represent the same stage in the gigas mutation. It will be seen that in type 22 there are 14 chromosomes, they have a tendency to come to lie in pairs. There are 7 pairs (I to VII, Plate XLVII, figures 1 and 2) as stated by Dombrowsky-sludsky [1927] for *Cicer arietinum* L., in gram type 22. But in gigas mutation it will be noticed that the number of

chromosomes is clearly sixteen and they are arranged in eight pairs (I to VIII, Plate XLVII, figures 3 and 4) in the metaphase stage.

Thus it is evident that the number of somatic chromosomes has increased by two in the gigas mutant over type 22 from which it has sprung up. The choromosome counts have also been made in some types of Kabuli gram, which appear to be the gigas forms of the small white-seeded gram [Dixit, 1932]. There also the diploid number of chromosomes is sixteen, as observed in the present gigas mutant. This difference of chromosome number by two is quite common in the different species of the genus Vicia, which is very closely allied to the genus Cicer. A perusal of the list of chromosome numbers in the natural order Leguminosae, shows that the diploid number in many of its sub-orders varies between 12, 14 and 16.

From the comparison of the somatic chromosomes in type 22 and its gigas mutant as seen the preparations in hand it is not possible to say definitely as to which is the extra pair of chromosomes in the mutant and how it has come.

(*b*) *Haploid number of chromosomes.*

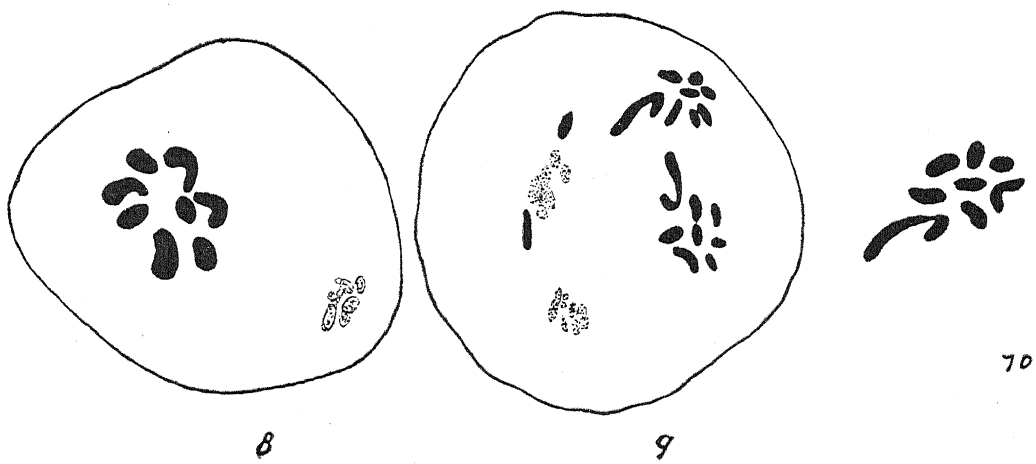
Material and methods.—The material for studying the haploid number of chromosomes consisted of pollen mother cells of the gigas mutant and type 22. An attempt was at first made to fix the young flower buds for this purpose. Material was fixed in different fixatives at different hours of the night and day, but the attempts were mostly futile. Only in one flower bud of the gigas mutation which was fixed in Carnoy's fluid at 10 a.m. some division stages were observed. But in this preparation there was too much shrinkage. The failure in fixing the proper stages in the flower buds seem to be due to the difficulty with which the fixatives penetrate through the floral appendages. To overcome this difficulty the Smear method was resorted to. Anther smears as described in my previous paper [Dixit, 1931] were prepared between 9 and 10 a.m., at which time the divisions seem to be most abundant. The slides were kept vertically in Allen's modification of Bouin's fluid for half an hour to three quarters of an hour, in a staining dish accommodating 12 slides. To wash away the fixative, slides were kept in changes of 40 per cent. alcohol, followed by grades of 30--20—15—10 and 5 per cent. alcohol. A few drops of saturated solution of lithium carbonate were added now and then to hasten the removal of picric acid. In this way slides were brought down to water and washed in running water for an hour. The slides were then stained in iron-alum-hœmatoxylin according to Kaufmann [1927] modification of Taylor's staining schedule. On examination of the slides it was found that there was some cloudiness in the preparations. It is perhaps due to the use of aqueous stain after the material has been killed and fixed by a fixative containing picric acid. In order to

PLATE XLVIII

GRAM TYPE 22



GIGAS MUTATION



overcome this difficulty it was thought advisable to stain in Delafield's hæmatoxylin which is an alcoholic stain. Some more smear preparations were made and fixed as described before in Allen's modification of Bouin's fluid. The schedule followed after fixing was different and is briefly given below :—

1. Kill and fix in Allen's modification of Bouin's fluid (picric acid saturated sol. in water 75 parts, formalin 15 parts, acetic acid 18 parts and a few crystals of urea.)	30 to 45 minutes.
2. Wash in 3 changes of 50 per cent. alcohol adding drops of saturated sol. of lithium carbonate.	10 minutes each.
3. 60 per cent. alcohol	5 minutes.
4. Delafield's hæmatoxylin	15 minutes.
5. 60 per cent. alcohol	Rinse to remove extra stain.
6. Acid alcohol (5 drops of HCl in 100 c.c. of 60 per cent. alcohol).	Differentiate to desired colour.
7. 70 per cent. alcohol	Change once or twice till no acidic reaction.
8. 80, 90 and absolute alcohol	5 minutes each.
9. Clove oil	1 or 2 minutes.
10. Xylol	Rinse to remove Clove oil completely.
11. Mount in Canada balsam.	

In this way about a dozen permanent slides can be prepared within 2 hours or so.

Chromosomes in preparations stained according to the above schedule take up a bright dark violet colour.

Chromosomes counts.—To confirm the results obtained from the study of chromosomes in the somatic cells—revealing that in the giant mutation the diploid number of chromosomes is sixteen, while in the parent type it is only fourteen—it was essential to find out in both the forms, the haploid number of chromosomes also.

From figures 5 and 6 (Plate XLVIII) it will be seen that the reduced number of chromosomes in gram type 22 is seven. This is the number noticed after the first and the second divisions of meiosis in pollen mother cells.

Figure 7 shows the number of chromosome in gigas mutation after the first reduction division. It is clearly eight. Figure 8 represents the stage after the second division is over, here also eight chromosomes can be seen.

In both the mutant and the type one chromosome (numbered 1) is distinctly longer than the rest as is seen in figures 9 and 10 where the haploid number of chromosomes in type 22 and the gigas mutant have been drawn on a greater magnification. It appears that one short chromosome is extra in the gigas mutation.

In short the haploid number of chromosomes in type 22 is seven and in the gigas mutant it is eight. This is in agreement with the result obtained for the somatic cells.

In one pollen mother cell of type 22 it was noticed that after the first reduction division there were eight chromosomes at one pole and only 6 at the other pole. This irregularity gives an inkling as to the method in which this mutation might have occurred, but the evidence is not enough to warrant any conclusions on the point.

V. Discussion.

The idea of the origin of new species as mutations was foreshadowed by Aristotle. Darwin although depending upon fluctuating variations as the material and natural selection as the force giving rise to new species, recognised the occasional occurrence of "sports." These however seemed to him to be so rare in nature as to offer no adequate basis for selection. Hugo De Vries in Holland and Bateson in England were however convinced that new species arise not by accumulation, through natural selection, of minute fluctuating variations, but by sudden appearance in one generation of fully formed new elementary species and they pronounced the mutation theory in the beginning of 20th century. Henceforwards other writers have expressed a variety of opinions concerning mutation from the extreme view that this is the only method of species origin, to the equally extreme denial that mutations have any evolutionary significance at all. However an impartial student of Nature will admit that though mutation does not furnish in itself a complete theory of evolution, but the daily accumulating data in favour of this theory indicates that mutation has played an important part in bringing about this multifarious diversity of earth's present flora and fauna.

Mutation as understood in the light of our present knowledge is defined by Gates [1930] as a change of any kind in germplasm, which having occurred will be transmitted by mitotic division from cell to cell and inherited, *i.e.*, the term mutation in a generic sense includes inherited changes of any kind in the germplasm ; various types of mutations such as trisomic, tetrasomic, polyploids, translocations and gene mutations being its different categories or classes.

The mutational origin of the present gigas gram culture 79 is indicated by the following facts :—

1. The gigas culture was derived from the seed of a single plant, which appeared suddenly and differed conspicuously in several characters (as described in the text) from the parent stock type 22.
2. Intermediate forms on selfing the mutant were not observed, which puts the idea of origin by re-combinations out of question.
3. The progenies of these plants remained uniform in their expression of new characters.

4. Comparison of the parent stock type 22 with any other gram type, with which it could have had an opportunity to hybridise does not reveal the source of distinguishing characters of the mutant.
5. The number of chromosomes in the gigas mutation is sixteen while in the parent type the number is fourteen, thus the change must have occurred in the germplasm—which having occurred was transmitted from cell to cell and inherited.

This type of change appears to have occurred quite frequently in the family Leguminosae—a perusal of the list of chromosome numbers in the different sub-orders of this family shows that the diploid number often fluctuates between 12-14 and 16. This is very common in the different species of the genus Vicia which is closely allied to the genus Cicer. A study of the chromosome numbers in the Kabuli gram types—which appear to be the gigas forms of small white seeded gram types revealed a similar change, diploid number being 16 in the former and 14 in the latter [Dixit, 1932].

This mutation in gram is not like the recorded cases of tetrasomic mutations with $2n+2$ chromosomes in matthiola [Howard, 1927], datura [Blakeslee and other] or oenothera in which the tetrasomic mutation plants are below the normal. Howard [1927] as the result of his studies on the mutation of *Matthiola incana* has remarked that an extra dose of one kind of chromosome reduces vigour and two doses are usually fatal, *i.e.*, mutation plants with $2n+1$ chromosomes are weak while those with $2n+2$ seldom survive. This is not the case in this gram mutation. Here the plants are more vigorous in every respect than the parent stock from which they have sprung up.

Surely the origin of a new species by mutation is proved by the above facts and the creation of the species Cicer gigas is quite logical. This species may become an outstanding commercial variety due to the size of its grains, like the mammoth forms of *Nicotiana tabacum* which arose as mutations from ordinary forms of tobacco and are now important commercial varieties in United States [Allard, 1919]. Or like the four varieties of Egyptian cotton which arose as single plant mutations and now form the basis of an important industry in that country [Kearney, 1918]. But the question as to how the mutation arose, under what circumstances the extra pair of chromosomes was formed and how it was formed, remains unanswered. Saying that it is the result of non-disjunction and might have originated as a seed formed by coming together of a pollen grain and an ovule with 8 chromosomes is a mere conjecture and a very rare possibility. To me the irregular distribution of chromosomes, 8 going to one pole and 6 to the other in some pollen mother cells seem to be the result of unnatural disturbance caused by chemical fixatives, etc., and may have no evolutionary importance in nature. What we want to find out

are the causes which produce new forms and, still more, to be able to produce the new forms ourselves by inducing such changes in the present ones. Until we have compassed this end, we have not reached our ultimate goal—namely a full knowledge of the origin of species.

VI. Summary.

1. There is an enormous increase in size of the giant mutation plants and all their organs over the parent type.

(*A*) *Vegetative.*

(*a*) Leaf of the mutant plant is about one and a half times longer than the leaf in type 22 and is characteristic in appearance.

(*b*) Length and breadth of the leaflet in the gigas mutant is more than twice as compared with the same in the parent type.

(*c*) Size of the stipules in the mutation is abnormally big and is about four times that of the stipules in type 22.

(*d*) Internode length is much longer in the mutation as compared with the type, *i.e.*, the number of leaves per branch in the mutant is smaller than that of type 22.

(*e*) Stem and roots are considerably thicker and more profusely branched in the mutation than the type. Their length is also greater in the former.

(*B*) *Floral.*

(*f*) The breadth of the standard is about one and a half times greater in the mutant as compared with the type; its length is also greater in the former.

(*g*) Length of the standard is less than its breadth in the mutant while in the type the length is greater than the breadth.

(*h*) Length of calyx in the mutation is one and a half times greater than that of type 22.

(*C*) *Pods.*

(*i*) Pods in the mutation are twice as big as the type both in their length and breadth.

(*j*) Shape of the pods is also characteristically different in the giant mutation.

(*k*) Percentage of sterile pods is about 21 per cent. in the mutation and 18 per cent. in the type.

(*D*) *Seeds.*

(*l*) Seeds of the mutant are twice as big as that of type 22. Their weight and volume is double that of the type and are also different in shape.

Cytological—(*a*) Diploid number of chromosomes in type 22 (the parent type) is fourteen while in the mutation the diploid number is sixteen.

(*b*) Haploid number of chromosomes in type 22 is seven, while in the mutation the haploid number is eight.

(*c*) One pollen mother cell was observed in the type where after the first reduction division there were 8 chromosomes on one pole and 6 chromosomes at the other.

VII. Conclusion.

1. The morphological, and cytological studies of this giant mutant indicate that it should be placed as a separate species of the genus Cicer and may be called as Cicer *gigas*.

2. New species arise at a single step in the genus Cicer as the result of chromosomal aberrations. The factors which are responsible for these chromosomal aberrations require further careful investigation.

REFERENCES.

Allard, H. A. (1919). *Amer. Nat.* **53**, p. 218-233.

Blakeslle, A. F. and Bellings, J. (1924). *J. Heredity* **15**, 194-206.

———————————— (1926). Proc. Nat. Acad. Xc XII ; 7-11.

Bremer, G. (1928). Rec. Tras. Bot. Neerl 25a, 82-91, abstract in Biol. Abs. Vol. 3, No. 4-6, 1929.

Davenport, C. B. Report Carnegie Inst. Wash. 20, 101-156.

Dixit, P. (1931). *Ind. J. Agric. Sci.* **1**, part IV, p. 419-433.

———— (1932). *Ind. J. Agric. Sci.* **2**, part IV, p. 385.

Denham, H. J. (1924). Ann. Bot., Vol. 38, 407-438.

Dombrowsky-sludsky, L. (1927). *J. Soc. Bot. Russie* **12**, 163-172. Ref. in Bot. Centrbl. N. F. 13 p. 66.

Gairdner, A. E. (1926). *J. Gen.* **16** : 341-351.

Gates, R. R. (1909). Arch. F. Zellforsch, 3, 525-552.

———— (1930). *Amer. Nat.* **65**, 97-120.

Gregory, R. P. (1909). Proc. Caml. Phil. Soc. 15, iii; 239-246.

————. (1914). Proc. Roy. Soc. Bot. LXXXVII, 484.

Howard, Howard and Khan, A. R. (1915). *Mem. Dept. Agric. Ind., Bot. Ser.* **7**, No. 6, 225-233.

Howard, B. F. (1927). *J. Heredity*, **18** 474—,483.

Kaufmann (1927). Stain Tchnology, 1927; Refer p. 119, Microscopical Technique edited by Mc-lung.

Keible, F. (1912). *J. Gen.* **2**, 163-188.

Kearney, T. H. (1918). *J. Heredity* **9**, 51-61.

Mol De (1922). Verslag Gewone vergadering, Wisen, Natuurk. Afd. der. kon. Ak V. Wet te Amsterdam, Deel XXXI.

——— (1923). Studia Mendelina, p. 161-167.

Shaw, F. J. F. (1929). Report Im. Econ. Botanist, 1928-29, p. 23.

Shaw, F. J. F. and Khan, A. Rahman (1931). *Mem. Dept. Agric. Ind., Bot. Ser.* **19**, No. 2, p. 27-47.

Sinoto, Y. (1925). *Bot. Mag. Tokyo*, **33**, 159-166.

Stompes, J. (1919). Zeithschr. indukt. Abstamm vereb 21 : 65-90 abstract Bot. Abs. Vol. IV, 778 entery.

Somme, A. S. (1930). *J. Gen.* **23**, No. 3, 447-507.

SELECTED ARTICLES

THE BIOLOGICAL CONTROL OF SUGAR CANE PESTS.*

BY

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[Reproduced by permission of the Controller of H. M. Stationery Office from the Report of the Proceedings of the Imperial Sugar Cane Research Conference, London, 1931.]

Although the cultivation of sugar cane is practically restricted to the tropics, it appears to be one of the most adaptable of the tropical crops, for it is grown with success, and under a great variety of ecological conditions, in regions differing not only in geographical position, fauna and flora, but also in climate and soil.

It is to this unusual adaptability of the sugar cane that its entomological misfortunes are partly due. Not many of the insects which attack it in its native home have, I think, migrated to the other regions in which it is now cultivated. On the other hand, in a great many of the areas into which sugar cane has been brought, the indigenous insects of the region have gradually spread from the native vegetation into the cane fields and have, in many cases, become serious pests. The fact that sugar cane is able to grow under such a variety of ecological situations exposes it to the attack of a great many different insects, so that the list of its pests is very long. To the same cause is due the fact, especially notable in the West Indies, that sugar cane pests tend to be restricted to certain colonies or regions. The pests of cacao, cotton and banana are much the same throughout the West Indies, because the conditions required for the growth of the plants are fairly well defined, but several of the most important insects attacking cane occur, or are at least of real economic importance, only in single colonies. Thus, in Trinidad, the most important pest of sugar cane is the frog hopper while in Barbados the most serious damage is caused by the *Diatræa* stalk-borers. In the East Indies, Australasia and Hawaii, of course, an entirely different set of insect pests of sugar cane is found. As I have already said, a good many of these insects seem to be rather limited in distribution, or, at least, the areas in which they are at present of economic significance, are relatively restricted. This does not mean, however, that the regions in which they exist, at present, are the only ones in which sugar cane can be grown in which they are likely to do damage. If they are transported into other areas many of them are capable of multiplying and becoming extremely

*Paper read at the Imperial Sugar Cane Research Conference, London, 1931.

injurious, especially if they are unaccompanied by the natural enemies which keep them in check in the native home. The improvement of means of transport in modern times is likely to produce an exchange of pests between the various sugar cane areas as time goes on, in spite of the quarantine regulations which now exist. As we know, this has already happened in the past and has, in fact, given rise to some of the worst sugar cane problems, as in Hawaii and in Mauritius, to take only two examples.

About 1897, the Delphacid bug *Perkinsiella saccharicida*, belonging to a Malayan genus, whose centre of distribution is probably New Guinea, was accidentally introduced into Hawaii. By 1902 it had increased to such an enormous extent that the sugar cane industry of the islands was threatened with destruction, and suggestions were seriously made that the sugar cane plantations be converted into cattle ranches.

Somewhere about 1905 the Lamellicorn beetle grub, *Phytalus smithi*, was accidentally imported from Barbados into Mauritius. It was not discovered until some six years after its introduction, by which time it had spread over about 3,000 acres, of which 300 acres were highly infested. An intensive campaign of control has been carried on against this species since that time, but in spite of this the beetle has continued to spread and now covers more than a quarter of the area planted with sugar cane. The measures adopted for its control have no doubt diminished the rate of spread to some extent, but there is no doubt that the numbers of the insect in the island are increasing and that the time will eventually come when the whole area is infested.

In the early part of the nineteenth century the Small Moth Borer, *Diatraea saccharalis*, was introduced into Louisiana, either from the West Indies or from South America; and since that time it has increased until it is now a serious pest. During the six-year period from 1912 to 1917 inclusive, during which observations were carried on in regard to this insect by the investigators of the United States Department of Agriculture, the average infestation was about 58 per cent. of all the canes in the infested area in Louisiana, which includes the whole sugar belt, or about 300,000 acres planted with sugar cane. The yearly damage in this area is estimated, by American authors, to result in a loss of sugar varying from 31,000 to 85,000 tons. The average monetary loss for the 300,000 acres reached the high figure of £1,400,000.

In recent years, of course, the movement of plant products from one country to another has been severely restricted and the careful inspection of imported material has been enforced. These measures have no doubt afforded some degree of protection and have, in a number of cases, prevented the entrance of injurious insects into new areas. It is, however, evident that they are not completely

efficacious. In spite of its elaborate and careful system of quarantine, the United States has been invaded during recent years by a number of extremely injurious pests from other parts of the world; such as the Japanese beetle, the European corn borer, and the Mediterranean fruit fly, which have caused immense losses and necessitated the expenditure of vast amounts of the public funds. It is, therefore, probable that the movement of the pests of cultivated crops in general, and of sugar cane among others, will continue, at least, to some extent and that the areas inhabited by a number of these pests will increase as time goes on.

It is an unfortunate fact that the method which is most suitable for sugar production at a low cost, that is to say, the system of large pure stands of cane as opposed to mixed agriculture, is especially suitable for the propagation of insect pests, to which, in general, systems of rotation are unfavourable. Such practices as ratooning, because they tend to leave residual colonies which are carried over to the next season, are also extremely favourable to the propagation and maintenance of injurious insects.

Furthermore, some of the most satisfactory methods used by the economic entomologist against pests attacking other crops, such as orchard fruits, vegetables, and so forth, are, in general, not applicable in the case of crops like cane; partly because of the difficulties of applying these remedial measures and partly because of the increase in the cost of production. Thus it is hardly conceivable that we shall ever be able to develop any methods of treating cane pests satisfactorily by spraying, in most areas. Such methods as flooding, burning, or dusting with arsenicals by means of aeroplanes, have sometimes been adopted and have occasionally given fairly satisfactory results, but it is evident that their use must be restricted to certain special cases.

On the other hand, the practices of estate cultivation, of growing cane continuously in large pure stands, year after year, on the same land, and even the practice of ratooning, are just as favourable for the specific insect enemies of the pests of sugar cane as they are for the pests themselves. Furthermore, while most methods of artificial control are difficult or impossible to apply to growing cane, especially in the early stages of its growth, the presence of the almost continuous mass of interlocking vegetation infested by its host is a positive advantage to the parasites and predators, or at all events causes no obstacle to their progress. As is well known, the most spectacular success obtained up to the present time in the methods of biological control have been secured under tropical conditions, one of the most recent being the control of the Levuana moth of coconuts in Fiji, through the admirable work of Dr. J. D. Tothill and his assistants. On general grounds, therefore, this method would appear to be fairly suitable as a means of attack against the insect pests of sugar cane.

As is well known, these theoretical considerations are well substantiated by the results that have been obtained in practice. I have said that in the Hawaiian Islands, owing to the attacks of the sugar cane leafhopper, the loss in sugar production was so great that about 1902 the conversion of some of the estates into cattle ranches was seriously suggested. It became evident that no artificial methods of control were likely to prove efficacious, so, on the advice of Dr. L. O. Howard, the entomologists of Hawaii decided to undertake the introduction of the insect enemies of the leafhoppers. After some three years' efforts, Perkins and Koebele succeeded in obtaining from Queensland and Fiji, two Hymenopterous parasites attacking the eggs of the leafhoppers. About eighteen months after the introduction had been carried out, the damage fell to about half of its original proportions, and during the following year 75 per cent. of the infested areas had been invaded by the beneficial insects. Nevertheless, outbreaks still continued to occur in certain districts and after much difficult and tedious exploratory work the late Dr. F. Muir succeeded in 1920, in introducing a small Capsid bug, *Cyrtorhinus mundulus*, which lives by sucking the eggs of the leafhoppers. This insect increased and spread throughout the infested areas and succeeded in completing the subjugation of the leafhopper, which has now become a problem of minor importance. The success obtained in this parasite campaign against the leafhopper rightly ranks as one of the most outstanding successes in biological control. It will, however, be noted, and I particularly wish to stress this point, that in spite of the exceptionally favourable conditions existing in the Hawaiian Islands, a real solution of the problem was not obtained without a tremendous amount of expensive exploration carried on during a period of almost twenty years. Nevertheless, the Hawaiian Planters' Association have felt that the expenditure of time and money has been amply justified by the results obtained.

Another important success obtained against the pests of sugar cane by the method of biological control, concerns the Sugar-cane Borer, which is a beetle existing in many parts of the regions in and about the Pacific. The damage to the plant is caused by the larva, which tunnels up and down the stalk. Insects of this type, which feed during the larval stages hidden in the tissues of plants, are, of course, rather inaccessible to parasitic attack during the greater part of the life-history and are, in fact, difficult to deal with by any method. Nevertheless, after a careful search throughout Japan, China and the Malayan Archipelago, the insect was ultimately found to be attacked in sago palms in Amboina by a Tachinid parasite, *Ceromasia sphenophori*. The collection of the parasite was begun in 1906, and in 1910, after great difficulties had been surmounted, it was successfully brought to Honolulu, where it was bred for a time in captivity and then liberated in the infested plantations. By the end of 1913 it had become firmly established in

thirty-nine different plantations and had already begun to produce a noticeable reduction, not only on the borers in the field, but also in the damage produced by them. On one plantation the number of adult beetles collected fell in two years from 27,000 to something like 1,500. This parasite is now being distributed in Queensland and appears to be giving satisfactory results.

Another remarkable success was obtained in the case of the Lamellicorn beetle, *Anomala orientalis*, which was probably introduced into the territory with potted plants some time before 1910. The introduction of the solitary wasp, *Scolia manilae*, obtained by Dr. Muir in the Philippines and taken to Honolulu in 1915, proved a very successful remedy for this pest. The spread of the beetle seems to have been arrested and although, at the present time, its distribution is increasing, the spread is extremely slow and the numbers remain small.

No results comparable to these have, as yet, been obtained in any of the British colonies in which sugar cane is grown. Some experiments in biological control have, of course, been carried out in certain regions. The parasitic wasp, *Tiphia parallella*, was successfully introduced into Mauritius from Barbados in the year 1916, in the hope that it would check the increase and spread of *Phytalus smithi*. It was recovered in the regions in which it was colonised the following year and has since spread over the whole of the infested region. In spite of this Phytalus has continued to increase and spread and although the parasite perhaps produces a slight diminution in the rate of multiplication of its host there is, in fact, no evidence that it is able to control the beetle. It should be added that another parasite belonging to the same group of insects, *Elis sexcincta*, was also introduced into Madagascar in the year 1916, but although the two species are said not to enter into competition, they are evidently not exerting a sufficient degree of control, at least at the present time. It is possible that when the beetle has reached the limits of its distribution and its population has become stable, the effect exerted by the parasites may be more apparent, but it seems more likely that other species, having different habits and, if possible a more rapid rate of multiplication, will have to be introduced into Mauritius. Arrangements for some work along these lines are now being made.

Attempts to use the parasites and predators of sugar cane insects, in order to bring about their control, have been made for many years past in the West Indies. These experiments have consisted, in the first place, in the transfer of various beneficial species from one area to another, and, in the second place, in the preservation, redistribution, and, in the last two or three years, the artificial multiplication of certain species of parasites, as well as certain fungous diseases, indigenous to the region. Up to the present, however, none of these experiments has given any practical results. One of the latest developments in this direction has been the

work on the common egg-parasite, *Trichogramma*. This parasite is present everywhere in the cane-fields, in fact, it is almost all over the world ; and it is frequently present in large numbers, producing the death of a high percentage of the eggs of the sugar-cane borer at certain periods of the year, especially towards the close of the growing season. During the period between the cutting of the mature cane and the appearance of new growth, the parasite, however, becomes relatively rare owing to the disappearance of its host, so that it is not usually found in appreciable numbers at the beginning of the new season. These facts have given rise to the hypothesis that if it were possible to supplement the initial population of this parasite in the cane, it might increase in numbers much more rapidly and bring the host insect under control before it succeeded in doing any appreciable damage. For a long time past, in many plantations in the West Indies, it has been the custom to collect, preserve and re-distribute eggs of the moth borers parasitised by *Trichogramma*, but it is only in the recent years that its artificial multiplication has been attempted. Largely through the efforts of some of the entomologists of California a very remarkable technique of rearing this parasite has been developed. By the use of a number of extremely ingenious devices it is now possible with the aid of relatively unskilled labour to breed a million individuals of the parasite a day at a cost of something under £3. As a result of the success obtained in the breeding work, attempts are now being made to utilise this parasite against a great many insect pests in many parts of the world, and in several places it is being bred and distributed in large numbers in the hope that it will prove an effective control of certain important pests of sugar cane, such as the small moth borer. Some of the entomologists who have been engaged in this work claim that through the artificial multiplication and distribution of *Trichogramma* a very perceptible decrease in damage has already been obtained. Unfortunately, however, the data published are, for the most part, open to criticism. In the first place, the difference in the percentage infestations and in the amount of damage in the years during which *Trichogramma* was distributed, as compared with the preceding periods, is much too great to be accounted for by the observed differences in egg parasitism in these two periods. In the second place, the results obtained from field experiments, specially designed to demonstrate the effects of liberating large numbers of *Trichogramma* in certain special areas, have been inconclusive ; in several instances, fields in which large numbers of parasites were released have actually presented towards the close of the season a lower percentage of parasitism than those in which no liberations were made.

We cannot definitely say at the present time that the mass breeding and distribution of *Trichogramma* is useless. But on the other hand, it must be admitted that none of the many field experiments carried on up to the present with this

parasite has given thoroughly satisfactory results. It would, therefore, be unwise to launch out on a large-scale programme of *Trichogramma* work until the projects at present under way in various parts of the world have been carried on a little longer.

The results obtained by measures of biological control in the West Indies are thus, up to the present, somewhat disappointing or, at least, inconclusive. Nevertheless, I am convinced that the possibilities of the method are very far from being exhausted within this area. Many of the important pests of sugar cane have probably originated on the mainland, while some have been transported from one island to the other, leaving their parasitic or predaceous enemies behind. It seems, therefore, provided that a thorough investigation of the area as a whole, together with an exploration of the South American mainland, can be carefully carried out, extremely valuable reeults might be obtained. The Imperial Institute of Entomology has, therefore, been carrying on since the autumn of 1928, with the aid of a special grant from the Empire Marketing Board, an intensive investigation of the possibilities of biological control in the West Indies and adjacent mainland areas, with special reference to the pests of sugar cane. This work has been carried on by Dr. J. G. Myers, and his preliminary report will, I think, be distributed at the close of this meeting. This is the first investigation covering the whole of the West Indian area that has yet been made with reference to the possibilities of biological control, and in view of this fact it has been considered desirable during the preliminary phases of the work, to deal with the important pests of practically all the important crops of the region. In view of this fact, and because of the difficulties of communication it has not yet been possible for Dr. Myers to settle down to any intensive large-scale work on particular problems. I have already pointed out that the outstanding successes obtained in regard to the pests of sugar cane in such regions as Hawaii, have been prepared and completed by many years of such investigation and exploration. It is not to be expected that successful results will follow more rapidly in this case, but, on the other hand, there is no reason to believe that conditions in the West Indies are less favourable to this type of work than they are in other sugar cane areas of the world.

As you know, the most important pest of sugar cane in the West Indian region as a whole are the Small Moth Borers (*Diatraea* spp.); only two of the numerous parasites attacking these insects in the various parts of their range have proved reasonably efficient, and of these *Lixophaga*, which is more effective, is apparently ecologically suited only to the conditions prevailing in the northern islands. It has accordingly been introduced into Barbados and Antigua, whence it may later be taken to St. Kitts and perhaps St. Lucia. Another valuable parasite of Diatraea, *Paratheresia*, which the United States are now introducing into Louisiana, occurs in all the areas in the West Indies, where it is likely to

thrive, excepting in the British Guiana province of Berbice and the island of St. Lucia. It may be advisable to introduce it into these two places and if so it will be done, but in the meantime the search for a more efficient parasite for Trinidad and British Guiana will be continued on the mainland. A large amount of data has been accumulated on the distribution of the Small Moth Borer and the complex of factors influencing it, and Dr. Myers has arranged, in conjunction with the local authorities and the entomologists of the West India area, for certain intensive investigations on the Small Moth Borers in certain parts of the region, particularly in British Guiana and in Antigua. Mr. H. E. Box has been appointed in the latter colony to undertake, under the supervision of Dr. Myers, a careful study of Diatraea in the island, and will make a careful study of the Trichogramma egg-parasite as a controlling agent. Before he left for the West Indies, Mr. Box carried on, during several months at Farnham Royal, a careful systematic study of the moths of the genus Diatraea, with the object of finding characters which facilitate the separation of these particular insects, and before his departure he published a very satisfactory monographic treatment of this subject. Mr. Cleare will carry on the Diatraea investigations in British Guiana. Both of these entomologists have had many years' experience with the problem of the Small Moth Borer.

The large moth borer of cane, *Castnia licoides*, is known as a serious pest only in Trinidad, since it is controlled by flooding in British Guiana. It is widespread and increasing in Trinidad and an effective parasite is badly needed. After an extensive search in a number of plants in Trinidad and the Guiana forests, the larvae of Castnia were eventually found fairly plentifully in *Heliconia bahai*, known in British Guiana as Wild Plantain, and collections carried out from this plant later on revealed the presence of an extremely promising Dipterous parasite. This was brought to Trinidad, but on account of the difficulty of obtaining material and of rearing the parasite this first introduction was not successful. It is hoped, however, to renew the attempt at an early date.

One of the most important pests in the West Indies is, of course, the Trinidad Cane Frog-hopper. A new and remarkably efficient frog-hopper parasite, which was found to be an important controlling agent of an allied frog-hopper, was studied by Dr. Myers in the hope that it would attack the sugar cane frog-hopper, but the experiments were unsuccessful. It is hoped that an effective parasite will be eventually discovered on the mainland. In the meantime a special investigation of the biology and natural control of the frog-hopper is being carried on by Mr. Pickles.

In Barbados, the cane root borer *Diaprepes abbreviatus*, is a very serious pest. An effective egg parasite (*Tetrastichus*) of two allied species has been studied in

Haiti and Montserrat, and a consignment of this has now been sent to the entomologist of Barbados.

Now that Dr. Myers has had an opportunity of studying the area as a whole and has become acquainted with West Indian conditions, we hope, providing funds for the continuation of the work are available, to take up work of a more intensive type upon the parasites of one after another of the most important pests of the region, particularly those of sugar cane. Excellent facilities for these studies have been provided by the authorities of the West Indies, and the hearty co-operation of the planters and officials has been freely granted. There is, therefore, every hope that it may eventually be possible, at least for certain of the more important pests of sugar cane, to duplicate in the West Indies the admirable results obtained by the use of the method of biological control with sugar cane pests in the other parts of the world, and thus relieve the planters of this region of part of the serious difficulties with which they are now struggling. The method of biological control has this great advantage that, when it can be successfully applied, it provides what is practically permanent relief at a relatively small initial cost. It is one of the few entomological methods to which the conditions of sugar cane cultivation offer no serious obstacle; and it seems from our experience in the past that it is destined to become increasingly important in tropical agriculture. We cannot, of course, expect to solve all of the entomological problems of the cane grower by the introduction of beneficial insects. Many of them will probably yield only to a modification of agricultural practice, or to the use of resistant varieties. Nevertheless, the method is one which seems to entail less modification of existing practices than those I have just mentioned. It seems, therefore, that in the present depressed state of the sugar industry it is on the method of biological control that we must cheifly rely. I would, therefore, venture to suggest to the Conference, the desirability of more intensive activity along these lines in the sugar-growing colonies of the Empire. No matter what may be the ultimate solution of the sugar problem, it seems certain that any method which will enable us permanently to diminish the cost of production without an addition to the burden of taxation, cannot fail to have a good and lasting effect upon the economics of the industry.

QUARANTINE AND THE SPREAD OF SUGAR CANE DISEASES.*

BY

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That part of the modern sugar industry which is based on the sugar cane has suffered severely in most territories from the introduction of serious diseases in foreign seed-cane due to lack of quarantine or its inadequacy. As a consequence, some territories prohibited or greatly restricted further introductions and focussed attention on the raising of their own seedlings. Of recent years, however, the demand of planters for trials under local conditions of new varieties (mostly seedlings) which had acquired a high reputation elsewhere and the need for enlarging the range of material available for selection in breeding work have modified this policy. New territories have, moreover, been opened up which are more dependent still upon introduced varieties. The danger of bringing in serious diseases has been, and is being, met by greatly improved quarantine methods, based upon a better knowledge of the disease, especially of those which have come to be regarded as major. It may, therefore, be of interest to draw attention to the six diseases which plant pathologists have recognised as major, and to list their present known distribution. The diseases in question are *Mosaic, Sereh, Fiji Disease, Gumming Disease, Leafscald, and Leafstripe.* Sereh, Fiji Disease and Leafstripe have a quite restricted distribution still, but Mosaic, Gumming Disease and Leafscald have spread widely, especially within the last twenty years. The present known distribution of the six diseases is shown in the table which follows:—

Mosaic	Fiji Disease	Sereh	Gumming	Leafscald	Leafstripe
West Indies	Queensland	Java	Queensland	Queensland	Queensland
United States	New South Wales	Formosa	New South Wales	New South Wales	Fiji
Peru	Fiji	...	Fiji	Fiji	Formosa
Argentine	New Guinea	...	Mauritius	Java	New Guinea
Surinum	Philippines	...	Reunion	Philippines	India†

* Paper read at the Imperial Sugar Cane Research Conference, London, 1931.

† Recorded in one locality in the present year (1931).

Mosaic	Fiji Disease	Sereh	Gumming	Leafscald	Leafstripe
Egypt . .	...	...	Brazil . .	Formosa .	...
Kenya . .	...	...	Colombia .	Mauritius .	...
Uganda . .	...	...	Porto Rico .	New Guinea .	...
South Africa .	...	...	Virgin Island (U. S. A.)	Hawaiian Islands	...
Reunion . .	...	...	Leeward Island (British West Indies).	...	...
India . .	...	...	St. Lucia .	...	...
Java . .	...	...	Barbados .	...	...
Queensland .	...	...	...	...	...
New South Wales	...	...	...	...	...
Fiji . . .	...	...	...	...	...
Hawaiian Islands	...	...	...	...	...
Formosa . .	...	...	...	...	...

These diseases affect directly all organs (systemic) except probably the true seed and are transmitted in the seed piece. With the exception of sereh, all cause characteristic discoloured stripes or streaks on the leaves which are distinctive for each. Specimens of leaves, preserved in the natural colours, showing the markings characteristic of these five major diseases, and of a number of minor diseases can be inspected at the Mycological Institute.

Mosaic Disease is an infectious chlorosis due to a virus which is transmitted by *Aphis maydis* Fitch. This insect transmits it also from sugar cane to maize and sorghum, and to a number of annual weed grasses. All thick tropical varieties (*Saccharum officinarum*) are susceptible to infection as well as a number of thin varieties (*S. barberi*), and the hybrids between such thin kinds and the thick canes. A number of semi-thin varieties (*S. sinense*), such as the Uba of Natal, do not take the infection, or do so very rarely. The disease occurs now in most sugar cane growing territories, British Guiana and Mauritius being outstanding exceptions. It can be controlled by careful seed-piece selection and roguing out young diseased plants, or by growing immune kinds such as Uba, or varieties highly resistant to infection, such as P. O. J. 2878, 2725, etc., or varieties which, although susceptible to infection, are very tolerant, such as some of the older P. O. J. series like 36, 213, 234.

Fiji Disease is of unknown cause, but behaves in the field as if it were infectious. It is readily recognised by the presence of pale narrow swellings or galls on the veins and midribs of the undersides of the leaves. All thick canes are susceptible to infection but vary in tolerance. The disease after causing severe losses was brought under control in Fiji by unremitting, careful seed-piece selection of the most resistant variety available (Badila) and by taking advantage of the fact, that the disease spreads less on poor than on rich land.

Gumming Disease is caused by the growth of a bacterium (*Bacterium vasculorum* (Cobb.) G. Smith), in the vessels of the vascular strands. The pathogen is transmitted from leaf to leaf during wind and rain, infection occurring at injuries especially at the abrasions caused by the prickles on the margins of the leaves. Infection may remain almost entirely restricted to the leaves, showing as characteristic narrow pale speckled stripes following the veins. In the more susceptible varieties, it may grow into the stems and fill the vessels with a yellow slime or gum; growth is checked or stopped and the gum may cause serious trouble in the factory. A number of varieties of thick canes are commercially resistant to Gumming, and the disease can be controlled by replacing the susceptible kinds with them. In territories where the disease is present new introductions or new seedlings should be tested in plots with kinds of known susceptibility or resistance, to decide if they are resistant enough for commercial planting.

Leafscald is also caused by the growth of a bacterium (*B. albilineans* Ashby) in the vessels of the vascular strands. This organism does not, however, produce a slime or gum, so that an infected cane when cut shows no ooze from the fibres. The disease occurs in a chronic and an acute form. In the chronic phase, long, narrow white stripes are present on the leaves, even on those just unfolding and the strands in the stem are reddened, especially in the nodes. As the cane approaches maturity, the eyes tend to produce short side shoots with diseased leaves showing white stripes, all the eyes on the stem frequently shooting in this way. In the acute phase, large canes when cold or dry weather causes a check to growth, wilt suddenly and wither up without showing signs of the disease either in the leaves or the stems; shoots springing later, however, from the base of the stem bear characteristic leafstripes. In the field, Leafscald behaves like an infectious disease although the mode of transmission is not definitely known. Varieties resistant or susceptible to Gumming are not necessarily so to Leafscald. The Hawaii seedling H. 109, is very susceptible to Gumming but is showing itself resistant to Leafscald in Australia. The disease has been controlled by means of resistant varieties.

Leafstripe is caused by a fungus (*Sclerospora sacchari* Miyake), one of the downy mildews. Shoots arising from diseased seed-pieces develop pallid leafstripes. The fructifications (conidiophores and conidia) are produced on the under sides of the

stripes during the night if a film of water is present due to dew or mist. The spores are carried by air currents to healthy leaves where germination and infection occur without delay. In favourable weather, the disease spreads rapidly in this way. The fungus advances into all organs of the cane, causing the stems to grow to an abnormal length, frequently twice the length of healthy canes; these long canes, however, contain little sugar so that heavy losses may result. The more fibrous varieties are resistant and the disease can be controlled by growing such as are commercially resistant; this involves loss as such varieties are often less productive than more susceptible kinds.

Sereh is a disease of unknown cause which behaves in the field as if it were infectious or contagious. The symptoms are liable to vary with the variety. The fibres are often reddened at the nodes and aerial roots may be produced in abundance. After topping, the upper eyes do not shoot as they would do in a healthy cane. In Java, all thick canes proved more or less susceptible, and the highly resistant hybrid seedlings which were bred were not productive enough for general planting. While control by seed-piece selection in the lowlands was difficult and uncertain, it proved quite feasible in the uplands, and by passing the cane through a descending succession of nurseries in the hills, healthy seed could be provided for the lowland cultivations every few years. This expensive though effective method of control, is now being gradually given up because the newer hybrid seedlings (P. O. J. 2878, 2725, etc.) are both highly productive and very resistant to the disease.

The problem of "latency".

Evidence has been obtained that the infection may remain "latent" or dormant in the cane for a number of months in these major diseases; no symptoms, characteristic of the disease, can be detected during the period of latency, but there is good ground for thinking that infection can be transmitted during this period when Gumming Disease, Leafscald, and Fiji Disease are present. Mr. D. S. North, pathologist to the Colonial Sugar Refining Company, has recorded an instance of latency in Fiji Disease. Cuttings carefully selected by him from seemingly healthy stools in Fiji, where the disease existed, were sent to two points in New South Wales where the disease was not present. The plants grew without showing symptoms for nine months when the disease began to show up in a number of them.

Recently, cuttings carefully selected in the Philippines, where Fiji Disease occurs, were sent to Java and planted in an isolated quarantine field. After some months the characteristic swellings or galls were detected on the leaves of some of the plants. In 1920, a supply of cuttings were sent from Formosa to a Japanese planting firm in the Philippines. Leafstripe was present in Formosa, but the cuttings

were carefully selected at the Government Experiment Station, and were believed to be healthy. They were allowed entry but periodically inspected by a pathologist during growth. No suspicious symptoms were detected on the plant cane, but in the ratoons, the characteristic leafstripes developed on many of the shoots. In this instance, the disease had remained latent for 12 months, passing through an entire generation in that condition. The disease was eventually eradicated by destroying all diseased and adjacent canes before infection was able to spread widely.

In Australia, North has observed many instances of this latent infection in Leaf-scald and Gumming Disease which may persist until the cane approaches maturity. In selecting seed-cane in infected districts, he recognises three classes of fields, (1) diseased, (2) unsafe and (3) safe. No seed-cane is taken from diseased fields nor from the ' unsafe ' fields adjacent to them, even if all the plants appear to be healthy. A field is recognized as " safe " only if it is at least a quarter of a mile from the nearest point of a diseased field.

The symptoms of Sereh Disease may also remain suppressed for long periods, especially in verieties which are resistant. The leaf symptoms of Mosaic may also be suppressed or obscure on very tolerant varieties, such as the older P. O. J. series (36, 213, 234) and some of the newer P. O. J. series. It appears to be generally true that infection remains latent or obscure longer the more resistant the variety is to these major diseases. It is evident, therefore that certificates of freedom from major diseases accompanying cuttings introduced from countries where one or more of them occur, must be accepted with reserve ; in making such introductions a prolonged period in quarantine in the receiving territory appears to be essential to meet the tendency of the diseases to remain latent.

Modern methods of quarantine.

Time does not allow of detailed reference to the methods in use. Valuable data can be found in the Proceedings of the First, Second and Third Congresses of the International Society of Sugar Cane Technologists, held in Hawaii (1924), Cuba (1927) and Java (1929), respectively. In the Proceedings of the Second Congress, E. W. Brandes described in detail, with illustrations, the quarantine glasshouse procedure in use at Washington in 1927, but begun some years earlier. The introduced cuttings are heated in water at a temperature of 50-51°C. for 15 minutes, then disinfected on the surface and planted in sterilised soil in large cans in an insect-proof glasshouse. They are grown here for a year and cuttings transferred to sterilised soil in concrete containers in a second house, and grown there to maturity before being released for propagation in sugar cane areas. At first, introductions from different countries were grown in separate compartments of the

glasshouse, but this was later abandoned and all were grown together. This procedure is open to criticism, and was apparently due to attention being focussed mainly on Mosaic; as the house was insect-proof the vector of this disease (*Aphis maydis*) was excluded, so that infection could not spread and diseased plants could be dealt with individually. In the case of Leafscald, Gumming and Fiji Disease where infection might be spread during the period of latency, safety would require that all plants in the house might have to be destroyed. In Hawaii, the Sugar Planters' Station put up a similar house [1924], but with the sides screened with insect-proof wire in place of glass, to avoid excessive temperatures. This house was provided to receive material which had been passed through the houses at Washington, as the quarantine there was not considered sufficient. Recently, the H. S. P. A. has removed its quarantine house to the island of Molokai, on which sugar cane is not grown commercially, and which is 40-50 miles from commercial cultivations. A house similar to that at Washington has been provided in Natal, and two glasshouses with wire-screened sides in Mauritius. The Colonial Sugar Refining Company has erected one also at Sydney, for introductions destined for their mills in New South Wales, Queensland and Fiji. They also plant introductions in private gardens near Sydney, a mile apart. The introductions from different countries are kept apart, and not more than three varieties grown together from the same source. Sydney is distant from the nearest cultivation, but the canes have been found to grow well in the open, in the sandy frost-free coastal district. The garden quarantine is maintained for two years. The Queensland Bureau of Sugar Experiment Stations, has adopted a similar garden procedure at Brisbane. In Formosa, Leafstripe, Leafscald, Sereh, Smut and Redrot were introduced in cuttings before quarantine was adopted in 1912. The Government has a quarantine field in Northern Formosa, outside the commercial sugar area, where introductions are grown for one season. Cuttings from this field are then brought to an isolated quarantine field in the tropical cane area, and grown for another season before being released.

In Java, quarantine was formerly quite lax, but few introductions were made. Fenced-off fields have now been made available (1929-30), in forest clearings on a mountainside at 2,500 feet, and distant by forest track three to four miles from the nearest native village. The introduced cuttings are planted seven feet apart to facilitate inspection during the early months of growth, and a laboratory is provided for the visiting pathologist who can remain, if necessary, several days. Reference has been made to the recent detection of Fiji Disease in one of these quarantine fields. A quarantine period of about two years has been rather generally adopted. If a glasshouse is available, the first year's growth is made in it, preferably to maturity, before transfer to a second house. In Hawaii, the second year is passed in an isolated quarantine field on Molokai as cane is not cultivated there

commercially. A first year in a glasshouse followed by a second year in an isolated quarantine field is to be recommended for the tropics, where conditions make it feasible, as it is desirable that the canes should be given an opportunity to develop in the open under normal conditions during a part of the quarantine period. In glasshouse quarantine, it is desirable that the introductions from different sources be grown in separate compartments : if this is not done, quarantine may tend to be unduly prolonged.

Introduction of true seed.

It is evident that much time and expense are involved in the introduction of varieties in the seed-piece, and always with some risk owing to latent infection and obscure symptoms (mosaic) even if quarantine is efficiently carried out. These difficulties would apply with much less force to introductions made as "true" seed. It is quite probable that none of the major diseases is carried in the true seed. No quarantine might be necessary, or only a short one. At the First Sugar Technologists Congress in Hawaii, in 1924, Brandes mentioned that true seed had been sent from India to Washington and given good germination. Germination of seed in the tassel after thirty days was also reported. Formosa was probably the first country to introduce true seed systematically. Owing to weather conditions, crossing is difficult, arrowing being uncertain and the pollen of some varieties, while fertile elsewhere, is often sterile there. Each season during the last nine years, a member of the staff of the Experiment Station has gone to Java and made crosses on a plantation controlled by a Japanese syndicate. The fuzz is sent to Formosa where it is fumigated and disinfected on arrival. Seven years' experience has shown that no disease has been introduced in this true seed. Recently, seed in the fuzz has been sent from Queensland and India to Hawaii. The seedlings raised from this seed, after it is threshed and disinfected, are grown under glasshouse quarantine of a few months and planted in an isolated quarantine field along with standard commercial varieties. Queensland is also introducing true seed from other countries and Java has brought in such seed from Hawaii. Reports on the liability of this seed to carry diseases will be awaited with much interests. This method may prove to be of much value if some central cane-breeding stations maintaining extensive collections of varieties can send away seed of known good parentage which can be depended on to give some good canes after selection. In this way territories, where production of good seed meets with difficulties, could raise seedlings from the introduced seed and make selections likely to be better suited to their conditions than seedlings selected elsewhere. It might, in large measure, obviate the need for introducing varieties in the seed-piece, except such as are very outstanding, and substantially lessen the risk of bringing in

diseases (see also in this connection A. Glendon Hill's Report on a visit to the Proefstation Oost-Java in March-April, 1930, E. M. B.-S. C.-33).

It may be mentioned, in conclusion, that no known cultivated canes are very resistant to all the major diseases. P. O. J. 2878, which now occupies over 90 per cent. of the estate area in Java, is very resistant to Sereh and very resistant to infection by Mosaic as well as very tolerant to it. It is susceptible to Leafscald in Java and Formosa and to Fiji Disease in the Philippines. It is also subject to some minor diseases such as Red Stripe (due to *Bacterium rubrilineans* (Lee et al.) Elliott) and a form of toprot (Pokkah Boeng). Two new diseases have, moreover, been reported recently on this seedling in Java, Ring Mosaic and Stipple. Ring Mosaic is expressed as brown concentric rings on the leaves and stems; it is a serious systemic disease, transmitted in the seed-piece, which renders the canes thin and short and liable to lodge easily. *Stipple*, which shows as small oval spots on the leaves, causes no loss of vigour and is probably of minor importance.

It may be inferred from what has been said that the old adage "more haste, less speed" may apply rather forcibly to the introduction of sugar cane varieties in the seed-piece, and that where adequate quarantine equipment has not been and cannot be provided, safety requires that such introductions be made indirectly, through some central point, where such quarantine has been or can be made available.

SELECTED REFERENCES.

North, D. S. The control of Sugar Cane Diseases. *Australian Sugar Journ.*, March, April, May, 1923. Also as Agricultural Report No. 6, Colonial Sugar Refining Co., Sydney, 1923.

——— Leaf Scald—a bacterial disease of Sugar Cane. Agricultural Report No. 8. Colonial Sugar Refining Co., Sydney, 1926.

——— Leaf Scald Disease of Sugar Cane and its control. Agricultural Report No. 9, C. S. R. Co., Sydney, 1929.

——— The dissemination of Gumming Disease. *Australian Sugar Journal*, August, 1927.

——— The bacterial Vascular diseases of Sugar Cane. Proc. Third Congress Internat. Soc. Sugar Cane Technologists, 142, 1930.

Brandes, E. W. A quarantine greenhouse for Sugar Cane. Proc. Second Congress Internat. Soc. Sugar Cane Technologists, 45, 1927.

——— Report of the Committee on Quarantine Practice, etc. Proc. Third Congress Internat. Soc. Sugar Cane Technologists, 103, 1930.

——— The Mosaic Disease of Sugar Cane and other grasses. *Bull. 829, U. S. Dep. Agric.* 1919.

Weston, W. H. A note relative to the recent appearance of the Sugar Cane Downy Mildew in the Philippines. *Phytopathology*, XI, 371, 1921.

Lyon, H. L. Three Major Cane Diseases: Mosaic, Sereh, and Fiji Disease. *Bull. Hawaiian Sugar Planters Assoc., Bot. Series*, III, pt. 1, 1921.

Ashby, S. F. Gumming Disease of Sugar Cane, etc. *Tropical Agriculture*, VI, 135, 1929.

Bell, A. F. A key for the field identification of Sugar Cane Diseases. *Bull. No. 2, Bureau of*

Sugar Experiment Stations, Queensland, 1929.

See also Proc. First, Second and Third Congresses, Internat. Soc. Sugar Cane Technologists—sections on Sugar Cane Diseases and Quarantines.

Addendum.

Undermentioned is the present known distribution of two important minor diseases:—

Red Stripe.	Streak (a virus disease).
Hawaiian Islands	South Africa
Queensland	Mauritius
(Fiji)	Reunion
United States	Egypt
Java	Madeira
Uganda	

NOTES

HYBRIDS BETWEEN NEW AND OLD WORLD COTTONS.

In a note dated January 14th, published in *Nature* of March 12th, Dr. Harland (Geneticist at the Cotton Research Station of the Empire Cotton Growing Corporation, Trinidad, B. W. I.) describes the successful production of a hybrid between Egyptian cotton (*G. barbadense*) and the red-flowered Indian tree cotton (*G. arboreum* var. *sanguinea*). When the occidental type was used as the female parent, a hybrid was obtained which, though sterile on the female side like the earlier crosses made by Zaitzeff, by Desai (between *G. herbaceum* and *G. hirsutum*) and by Harland himself possessed a few functional pollen grains and could be crossed back on to *G. barbadense*. Of the "back cross" eight healthy plants were raised to maturity. Further back crosses were made between these eight hybrids, and *G. barbadense* and some plants were obtained which were fertile on both male and female sides.

RESEARCH IN PROGRESS IN THE BRITISH EMPIRE.

The Imperial Bureau of Plant Genetics (for crops other than herbage), School of Agriculture, Cambridge, has just issued an "Account of the Research in Progress in the British Empire". The history and object of this publication is best described in the compilers' own introduction to this valuable bibliography which is, therefore, quoted in full.

"On March 12th, 1930, the Imperial Bureau of Plant Genetics issued a questionnaire to the Official Correspondents in the various countries which the Bureau was intended to serve. In addition to the Official Correspondents, a number of research officers and institutions known by the Staff of the Bureau to be engaged in plant breeding also received the questionnaire. One of the first aims of the Bureau was to obtain full information of the nature of the work being carried out in each research department of the empire. Only by so doing would the Bureau be in a position to put workers in one part of the empire in contact with workers in other parts interested in similar or allied fields. The Bureau was therefore in the first place dependent upon the response of the correspondents; most of them realized this and I should like to take this opportunity of thanking these correspondents for the care and promptitude of their replies.

When the replies to these questions began to come in, it became evident what a vast amount of material, original, authoritative and often not elsewhere available, was being collected. The desirability of making this material available, of so making known what are the problems of each country, what methods are in use to solve them, with an indication of the results so far obtained, led to the present publication. It is in fact a compilation of the information received from correspondents in reply to my questionnaire. The compilation has not been easy. Some replies were

much fuller than others. Wherever possible the information where scanty has been supplemented by reference to the Bureau files, to annual reports and other official documents. In spite of these efforts the compilers are not yet sure that everything of importance has been included, or that the space allotted to each piece of work strictly corresponds to the relative importance of the work It has perforce been dictated by the degree of completeness of the replies. We hope, however, that readers will treat us with consideration in such respects and it is earnestly hoped that any serious omissions will be immediately notified.

We frankly expect everyone who reads this work with care to be surprised at the volume of research on such diverse subjects which is in progress in the empire. We hope that it will introduce each reader to some new piece of research of interest to him and about which he has not previously known. We hope further that when such is the case we shall have an immediate application for more details of that piece of work or that we shall be able to put our correspondent into direct touch with those engaged in it. With the possibility of this in mind we have omitted all but what we considered the most important details.

The work is arranged under crops, these being classified according to the Universal Decimal Classification, the explanation of which has been the subject of a previous memoir. Within each crop the work is sub-divided according to the country in which it is being done. The countries are also arranged according to the Universal Decimal Classification. For convenience of reference an index is given of both subjects and countries. By means of these indexes it is possible to find what work is in progress on a particular crop regardless of country, what work is going on in a particular country regardless of crop, or finally the work in a given crop in a given country".

Free copies have been sent to all those on the Bureau's free distribution list, and this secures approximately two copies for each Department of Agriculture. Additional copies can be obtained from the Bureau, price 3*s*. 6*d*.

The Official Correspondent in India for the Imperial Bureau of Plant Genetics (Agricultural Expert, Imperial Council of Agricultural Research) would be glad to receive at any time amendments and additions to this publication for forwarding to the Bureau for inclusion in any subsequent edition.

INTERSPECIFIC AND INTERGENERIC HYBRIDIZATION IN RELATION TO PLANT-BREEDING.

This bibliography issued by the Imperial Bureau of Plant Genetics, School of Agriculture, Cambridge, in February 1932 is really in the nature of a technical communication giving a very general survey of the literature on this intricate subject and an indication of its main features. The discussion is sufficiently full to be of considerable value and interest to all plant-breeders. Copies have already been supplied to those on the Bureau's free distribution list, which secures approximately two copies for each Agricultural Department; additional copies can be obtained from the Bureau, price 2 shillings.

THE SIXTH INTERNATIONAL BOTANICAL CONGRESS.

According to a decision by the Fifth International Botanical Congress at Cambridge in 1930, the Sixth Congress will be held in Holland in 1935. An Executive

Committee has been formed, President of which is Professor Dr. F. A. F. C. Went (Utrecht), while Professor Dr. J. C. Schoute (Groningen) will act as Vice-President, Dr. W. C. de Leeuw (Bilthoven) as Treasurer and Dr. M. J. Sirks (Wageningen) as Secretary. The Committee has decided that the Sixth Congress will meet at Amsterdam, September 9th-14th, 1935. Scientific societies are kindly requested to reckon with these data in planning their own meetings.

THE MAYNARD GANGA RAM PRIZE, 1932.

Applications are invited for the 'The Maynard Ganga Ram Prize' of the value of Rs. 3,000 which will be awarded for a discovery, or an invention, or a new practical method tending to increase agricultural production in the Punjab on a paying basis. The prize is open to all irrepective of caste, creed or nationality and Government servants are also eligible for it. Some part of the discovery, invention, etc., must be the result of work done after the prize was founded in 1925. The Managing Committee reserves to itself the right of withholding or postponing the prize, if no satisfactory achievement is reported to it. All applications in competition for the next award should reach the Director of Agriculture, Punjab, Lahore, on or before the 31st December, 1932.

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A complete list of the publications of the Imperial Department of Agriculture in India can be obtained on application from the above-mentioned.

The following are the publications of the last two years:—

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Review of Agricultural Operations in India, 1926-27. Price, Rs. 2 or 3*s.* 6*d.*

Review of Agricultural Operations in India, 1927-28. Price, Rs. 2-6 or 4*s.* 3*d.*

Review of Agricultural Operations in India, 1928-29. Price, Rs. 3-2 or 5*s.* 6*d.*

MEMOIRS OF THE DEPARTMENT OF AGRICULTURE IN INDIA.

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ORIGINAL ARTICLES

CARBON DIOXIDE ASSIMILATION OF THE LEAVES OF THE RICE PLANT, *ORYZA SATIVA* L.

BY

R. H. DASTUR,

AND

J. J. CHINOY,

Botany Department, Royal Institute of Science, Bombay.

(Received for publication on the 10th June 1932)

(With 5 text-figs.)

The absorption and assimilation of nutrients by the rice plant has been the subject of investigations with the agriculturists for some time past with a view to determine the time and the nature of the fertilizer to be given to the plant in order to secure the maximum yield with a mininum expenditure of manures. The determination of chemical composition of the rice plant at different stages of growth is the chief method employed in these investigations.

Absorption of various inorganic salts has been studied and a large amount of useful information is now available, on account of the valuable work of Miyake [1914] and others, about the quantities of different substances, such as potassium, phosporus and nitrogen absorbed by the rice plant at different stages of growth. On chemical analysis of the plants, it was found that different salts were needed at different stages of growth.

Gile and Carrero [1915] worked on the absorption of iron salts and found that they were only slightly absorbed by the rice plant. They determined the iron content of the plant at different stages of growth and showed that the percentage of iron in green leaves and straw decreased with age but remained constant in the ash of the whole plant after 26 days.

Kelley and Thompson [1910] determined the composition of the rice plant at different stages of growth when treated with different fertilizers and determined the

nature of carbohydrates formed in the plant. Kelley [1911] further showed that ammonium sulphate was a better fertilizer than the nitrates of different metals and showed the best time for manuring the plants.

The assimilation of nutrients by the rice plant has also been studied in India by Sen [1916] and by Sahasrabuddhe [1928]. The latter author also determined the chemical composition of the rice plant (Kolamba variety, 42) commonly cultivated in the Colaba district with a view to determine the assimilatory activities of the plant at different stages of growth. He determined the quantities of ash, albuminoids, soluble carbohydrates, woody fibres and oil during its life-cycle, at first at regular intervals of 15 days and later at different stages of reproductive activity such as flowering period, milk-stage, half-ripe stage and full-ripe stage; and from the quantities of the different substances found at different stages he concluded that the assimilatory activity of the plant continues up to the ripening period and there is a rise in the assimilatory activity at the flowering stage and at the milk-stage. He therefore recommends to manure the plant at the two last stages.

The investigations of the abovementioned writers, though they give the general idea of the total assimilatory activity of the rice plant at different stages, they do not tell us anything about the photosynthetic activities of the leaves in the corresponding stages. The soluble carbohydrate figures given by Sahasrabuddhe [1928] and others are obtained indirectly by subtracting from 100 the amounts of ether extracts, albuminoids, woody fibre and ash, but these figures convey nothing about the rate of photosynthetic activities of the leaves. The other assimilatory activities of the plant follow the photosynthetic process of the leaves as carbohydrates are required in the synthesis of proteins and for the production of other substances. As the total amounts of soluble carbohydrates present in the leaves do not give us a measure of their photosynthetic power at a particular stage, it is necessary to measure separately the rate of photosynthesis in leaves at different stages. It would then be possible to determine the periods of maximum photosynthetic activities of the leaves and as other processes depend upon or follow the photosynthetic process, the information thus obtained may be of use in determining the time of manuring the plants when the photosynthetic activity of their leaves is at its highest pitch. It would then also be possible to see if the periods of maximum assimilatory activities as obtained by Sahasrabuddhe [1928] coincided with the periods of maximum photosynthetic activity of the leaves of the rice plant. As no such measurements so far as the authors are aware have been made of the photosynthetic activity of the leaves of the rice plant and as the results are likely to prove of interest, this investigation on the carbon dioxide assimilation of the leaves of the rice plant is undertaken.

INVESTIGATION.

The photosynthetic rate of the leaves of the rice plant is measured in two different ways. (1) It is measured by measuring the difference in the quantities of different carbohydrates present in the leaves analysed early in the morning before sunrise and in the evening before sunset, at different stages of growth of the rice plant. This method has got evident objections, as respiration and translocation of carbohydrate material introduce errors in investigations. However, it is sufficiently accurate to give a general idea, if not a definitely accurate one, of the photosynthetic activities of the leaves at different stages of growth. (2) The rate of photosynthesis is measured by finding out the quantity of carbon dioxide absorbed by unit leaf area in unit time under controlled conditions of experimentations.

Determination of carbohydrates in leaves.—The extraction and estimation of carbohydrates in leaves has proved a difficult problem owing to the presence of enzymes in the leaves and on account of very minute quantities of carbohydrates present in them. Various attempts were made to measure the carbohydrate contents of leaves but they were all open to grave objections on account of apparent defects in the technique of the methods employed. Great advance was made, however, in the technique of estimating carbohydrates by Brown and Morris [1893] when they first attempted the sugar analysis of the leaves of *Tropaeolum majus*. The methods employed in the estimation of carbohydrates were purely chemical, depending upon osazone tests and copper reducing power of sugars before and after hydrolysis. Similar methods were employed by Park [1911], Lewis and Tuttle [1920] and many others. Davis, Daish and Sawyer [1913] were the first to point out the defects in the methods employed by previous workers for extracting the leaves and made various improvements. Brown and Morris [1893] dried the leaves in an oven. This introduced an error in the estimations of the respective quantities of carbohydrates present in the leaves, as during the slow process of drying the enzymes acted upon the sugar and brought about a change in their respective amounts. Maltose is normally absent in the leaves but is found to be present in the leaves in the experiments of Brown and Morris [1893] which is due to the action of the enzyme known as maltase on the starch present in the leaves. This error was avoided by Davis, Daish and Sawyer [1913] by dropping the leaves in boiling alcohol containing about one per cent. of ammonium hydroxide and thus destroying the enzymes instantaneously. Addition of a little ammonia to the alcohol facilitates the penetration of the latter into the tissues of the leaves and killing of the enzymes as soon as they come in contact with it. The same authors have greatly improved upon the technique of separating other foreign substances such as gums, resins, tannins and others from the sugar solution,

especially by the use of basic lead acetate. They have also improved upon the methods of inversion of cane sugar by 10 per cent. citric acid and of the hydrolysis of starch into maltose and dextrose by the use of taka-diastase at 38°-40°C. They also made polarimetric estimations of the carbohydrates at a constant temperature with a monochromatic light from a sodium lamp.

The same methods of extracting and separating the carbohydrates from different foreign material are employed in this investigation with certain modifications and precautions according as the nature of the assimilating material and the local conditions made it imperative. For estimating the carbohydrates, colorimetric method first used by Folin and Wu [1918], Calvert [1923, 1924], and recently improved upon by Dastur and Samant, in this laboratory, is used.

It is noticed that the method of extraction of sugars used by Davis, Daish and Sawyer [1913] is not exactly applicable to the extraction of sugars from the leaves of the rice plant. In order to extract the sugars completely it is necessary to extract the leaves with alcohol for nearly 100 hours, otherwise much of the sugars remain unextracted. One year's work on the carbohydrate analysis had to be discarded on account of this important point being unnoticed before.

Similarly the method of estimating sugars improved upon by Dastur and Samant had to be greatly modified as experience was gained and the whole method is now so perfected that it can be used as a standard method for estimating carbohydrates present in a small sample of plant material. It is hoped to publish the whole method of extraction and estimation of carbohydrates in a separate paper.

Method.

The rice seedlings (Kolamba variety 42) were obtained from Karjat and transplanted in a special plot of ground in the College Garden. Transplantation was carried out in the usual way on 23rd of July 1931. A week was allowed to elapse for the plants to get a firm hold in the soil. The first reading for the measurement of photosynthetic activity was taken on the 1st August.

Leaves for extraction were gathered from the same bed for the morning as well as evening readings. Great care was taken to select leaves of all ages and leaves of only half the bunch were plucked for the morning reading, the other half was left for the evening reading. As soon as the leaves were plucked from a certain part of the bed it was marked off and no samples were taken from that portion again.

The leaves were removed without any loss of time to the laboratory and 50 grms. were taken for extraction. After weighing, the leaves were quickly cut into small pieces and were introduced into a round-bottomed flask containing

boiling alcohol to which a little ammonia was added as recommended by Davis, Daish and Sawyer [1913]. The sugars and starch are extracted and estimated according to the methods mentioned above. The following tables (I—V) give the results of the carbohydrate analysis of the leaves of the rice plant taken in the morning at 6 A.M. and in the evening at 6 P.M.

TABLE I.

The different carbohydrates present in the leaves in the morning.

Date	Hexoses in 100 grms. of dry weight of leaves in grms.	Cane sugar as hexoses per 100 grms. of dry weight of leaves in grms.	Starch as hexoses per 100 grms. of dry weight of leaves in grms.
1931			
1st August	0·0335	1·9785	0·1255
8th ,,	0·0233	2·3177	0·1312
17th ,,	0·0520	2·8960	0·2675
25th ,,	*Nil*	2·4730	0·2601
3rd September	0·0313	3·3677	0·2556
11th ,,	*Nil*	1·9770	0·2173
21st ,,	*Nil*	3·9440	0·1998
30th ,,	*Nil*	3·1370	0·1840
9th October	*Nil*	2·9870	0·1484
18th ,,	0·0286	5·4604	0·1248
28th ,,	0·0242	2·8858	0·1093

TABLE II.

The different carbohydrates present in the leaves in the evening at 6 p.m.

Date	Hexoses in 100 grms. of dry weight of leaves in grms.	Cane sugar as hexoses per 100 grms. of dry weight of leaves in grms.	Starch as hexoses per 100 grms. of dry weight of leaves in grms.
1931			
1st August	0·0477	6·7003	0·4540
8th ,,	0·0529	11·8771	0·5572
17th ,,	0·1647	13·0953	0·7129
25th ,,	0·1060	11·1740	0·8198
3rd September	0·0455	9·6395	0·7958
11th ,,	0·0258	8·6242	0·5675
21st ,,	0·0272	6·2178	0·5789
30th ,,	0·0286	5·0034	0·3922
9th October	0·0639	13·0561	1·0725
18th ,,	0·2271	18·6279	1·9381
28th ,,	0·0379	6·2701	0·5754

TABLE III.

Quantities of different carbohydrates formed in the leaves in ten hours.

Date	Hexoses per 100 grms. of dry leaf material in grms. (difference)	Cane sugar as hexoses per 100 grms. of dry leaf material in grms. (difference)	Starch as hexoses per 100 grms. of dry leaf material in grms. (difference)
1931			
1st August	0·0142	4·7218	0·3285
8th ,,	0·0296	9·5594	0·4260
17th ,,	0·1127	9·1993	0·4454
25th ,,	0·1060	8·7010	0·5597
3rd September	0·0142	6·2718	0·5402
11th ,,	0·0258	6·6472	0·3502
21st ,,	0·0272	2·2738	0·3791
30th ,,	0·0286	1·8664	0·2078
9th October	0·0639	10·0691	0·9241
18th ,,	0·1985	13·1675	1·8133
28th ,,	0·0137	3·3843	0·4661

TABLE IV.

Total carbohydrates present in the leaves in the morning.

Date	Total carbohydrates as percentage of dry weight of the leaves in grms.	Total carbohydrates as percentage of fresh weight of leaves in grms.
1931		
1st August	2·1375	0·3547
8th ,,	2·4722	0·4456
17th ,,	3·2155	0·6653
25th ,,	2·7331	0·5189
3rd September	3·6546	0·8304
11th ,,	2·1943	0·5453
21st ,,	4·1438	0·9781
30th ,,	3·3210	0·8634
9th October	3·1354	0·8178
18th ,,	5·6138	1·6241
28th ,,	3·0193	0·9143

TABLE V.

Total carbohydrates present in the leaves in the evening at 6 p.m.

Date	Total carbohydrates as percentage of dry weight of the leaves in grms.	Total carbohydrates as percentage of fresh weight of leaves in grms.
1931		
1st August	7·2020	1·3769
8th ,,	12·4872	2·4649
17th ,,	13·9729	2·8810
25th ,,	12·0998	2·4822
3rd September	10·4808	2·4479
11th ,,	9·2175	2·2751
21st ,,	6·8239	1·7964
30th ,,	5·4242	1·3884
9th October	14·1925	3·9288
18th ,,	20·7931	5·9155
28th ,,	6·8834	2·2225

TABLE VI.

Total carbohydrates formed in the leaves in ten hours.

Date	Total carbohydrates as percentage of the dry weight of leaves in grms.	Total carbohydrates as percentage of the fresh weight of leaves in grms.
1931		
1st August	5·0645	1·0222
8th ,,	10·0150	2·0193
17th ,,	10·7574	2·2157
25th ,,	9·3667	1·9633
3rd September	6·8262	1·6175
11th ,,	7·0232	1·7298
21st ,,	2·6801	0·8183
30th ,,	2·1032	0·5250
9th October	11·0571	3·1110
18th ,,	15·1793	4·2914
28th ,,	3·8641	1·3082

Table I gives the quantities of hexoses, cane sugar and starch present in the leaves in the morning. The cane sugar and starch values are given in equivalent hexoses in all the tables. It is evident from Table II that there is certain amount of carbohydrate still left in the leaves in the morning and the quantity of cane sugar present is in larger quantities than hexoses and starch. The quantity of hexoses present in the morning is very small and is totally absent on some days.

Table II gives the values of the three carbohydrates present in the evening in the leaves of the rice plant. Again the quantity of cane sugar present on each day far exceeds the other carbohydrates. There are two periods when the quantity of cane sugar reaches its maximum values, the first period being in August and the second in October at the time of flowering. The quantity of starch present is very little in comparison with that of the cane sugar. In August and in October the starch values, like the cane sugar values, are higher than those obtained on other days. A big depression in the quantity of carbohydrates in September is an unique feature of these results.

Table III gives the quantities of carbohydrates formed in the leaves in ten hours and obtained by deducting the values of the three carbohydrates present in the morning samples from the corresponding values present in the evening samples. The results show the same features as discussed above supporting the same conclusions.

If the results are studied as the total carbohydrates as percentages of either dry or fresh weights present in morning or evening samples or as the total carbohydrates formed in ten hours, exactly the same conclusions are arrived at. The total carbohydrate values for the morning and evening samples of leaves and formed in leaves in ten hours are given in Tables IV, V and VI. The total carbohydrate values given in these tables are calculated both as percentage of fresh and dry weights. It is clear from the results obtained that the photosynthetic activity increases after transplantation and reaches its first maximum in August. There is a depression in the photosynthetic activity of the plant in September and it reaches its second maximum in October at the time of flowering and then it falls towards the end of October.

The graphs showing the photosynthetic activity of the rice plant during the season are given in Figure 1. The total carbohydrate values in the morning and evening samples and formed in ten hours are given as percentage of fresh weights.

Similarly the graphs for the cane sugar values in the morning and evening samples and formed in ten hours are given in Figure 2.

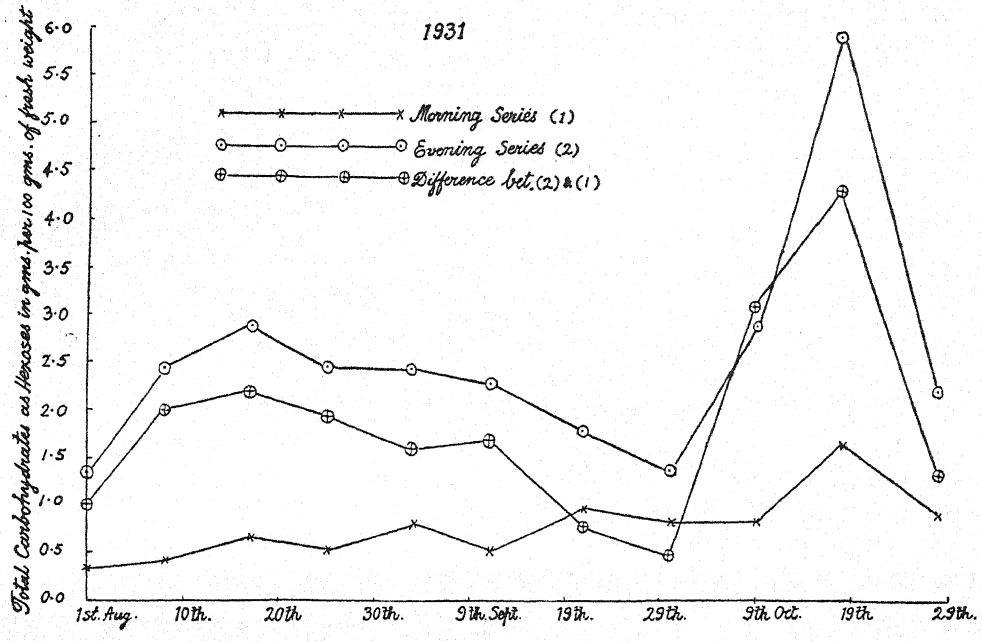


Fig. 1. Total carbohydrates as hexoses in grams per 100 grams of fresh weight of the leaves present in the morning, in the evening and formed in ten hours in 1931.

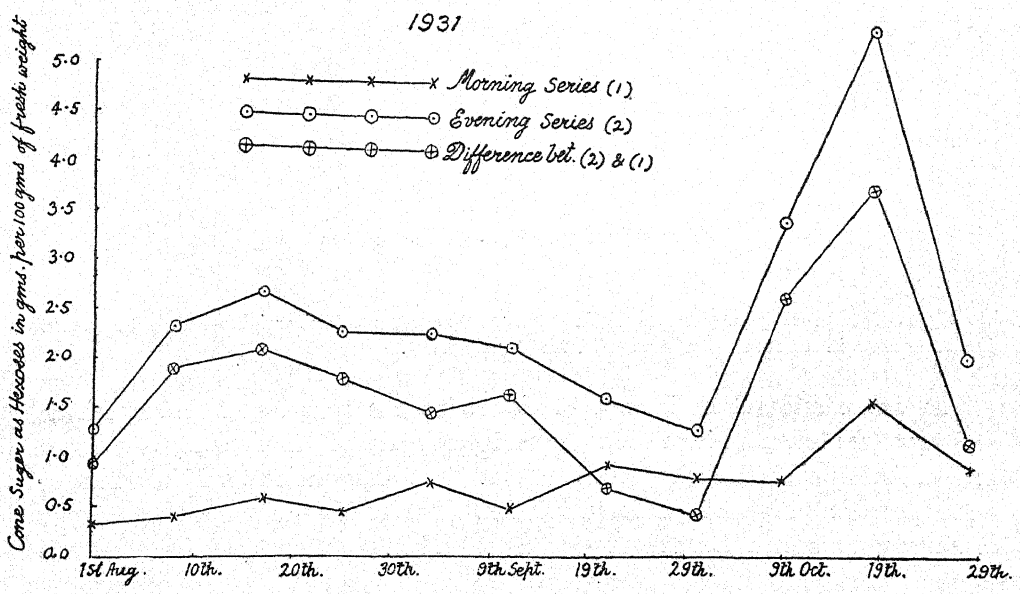


Fig. 2. Total cane sugar as hexoses in grams per 100 grams of fresh weight of the leaves present in the morning, in the evening and formed in ten hours in 1931.

The carbohydrate analysis of the leaves in the same manner described above was made again in the previous year and the results showed the same features. As the number of readings taken in 1930 was small, they are not given in detail here but the graphs showing the photosynthetic activity as determined by the total carbohydrate values present in the morning and evening samples and formed in ten hours are given in Figure 3. The curves of the photosynthetic activity during the season obtained for both the years are identical.

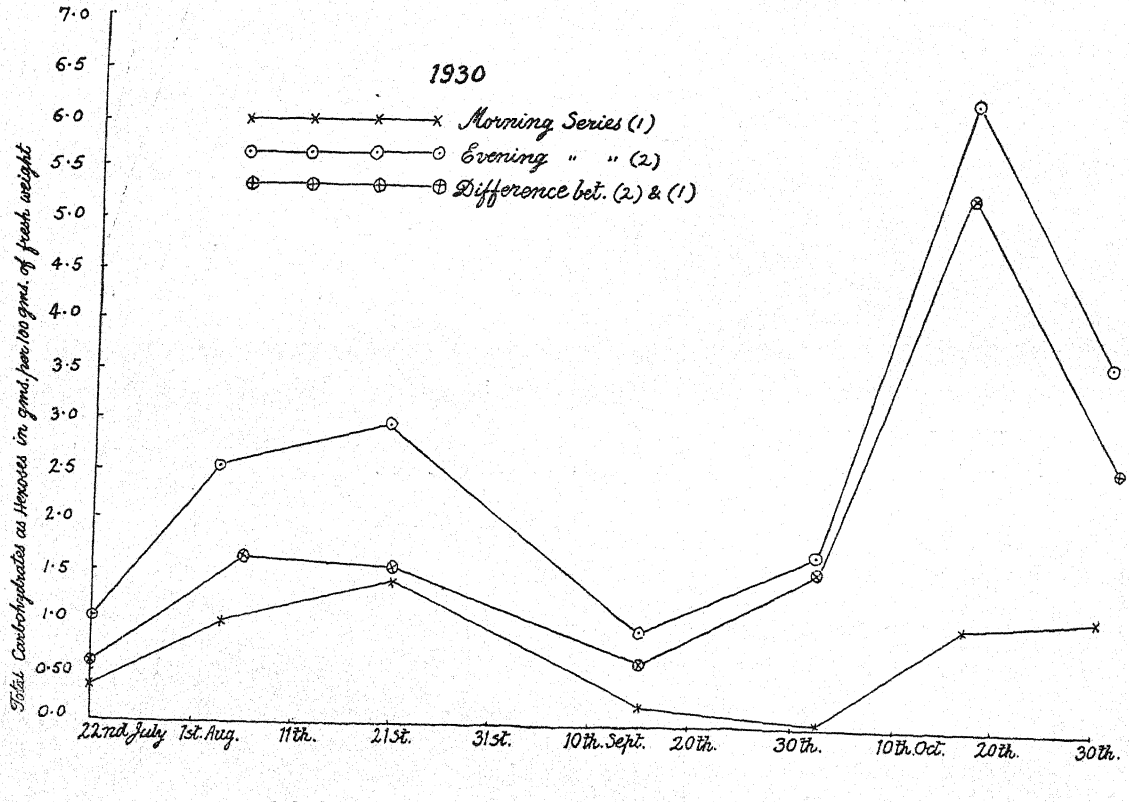


Fig. 3. Total carbohydrates as hexoses in grams per 100 grams of fresh weight of the leaves present in the morning, in the evening and formed in ten hours in 1930.

It was considered of interest to determine the progress of photosynthetic activity of the leaves during the day by taking samples of leaves every six hours and analysing their carbohydrate contents. The best period would be when the photosynthetic activity is at its maximum. One such series of sugar analysis of leaves was made on the 17th August, the second on the 21st September, and the

third on the 18th October. The samples were taken at 6 A.M., 12 noon, 6 P.M. and 12 midnight.

TABLE VII.

Results of Sugar analyses of leaves on 17th August 1931. (Rain for two hours at 12 noon. Temp. 27°-28° C.)

———	6 A.M.	12 Noon	6 P.M.	12 Midnight
Hexoses	0·0520	0·2332	0·1647	*Nil.*
Cane sugar	2·8960	13·1268	13·0953	8·7090
Starch	0·2675	0·4969	0·7129	0·3668
Total carbohydrates as hexoses .	3·2155	13·8569	13·9729	9·0758

The quantities of different carbohydrates increase at 12 noon and remain constant till 6 P.M. except for a slight decline in cane sugar and increase in starch. At midnight the cane sugar value has fallen to 8·7090 grms. while the starch value is much reduced. The fall in cane sugar indicates that cane sugar is translocated as such, as there is no corresponding increase in the hexoses which on the contrary are totally absent. From the constant value of cane sugar obtained at 12 noon and 6 P.M. it is difficult to say whether the photosynthetic process goes on vigorously or it is checked in its rapidity on account of the accumulation of photosynthetic products. It is also reasonable to expect that translocation goes on during the day time and the constant values of carbohydrates indicate the maximum capacity of leaves to store up the photosynthetic products. If the latter is the case, the rate of the photosynthetic process is generally influenced by the speed of translocation of the photosynthetic products.

The same features were noticed in the second series of sugar analysis carried out on the 21st September.

TABLE VIII.

Results of Sugar analyses of leaves on 21st September 1931.

———	6 A.M.	12 Noon	6 P.M.	12 Midnight
Hexoses	*Nil.*	0·0257	0·0272	0·0240
Cane sugar	3·9440	6·9923	6·2178	4·8490
Starch	0·1998	0·5548	0·5789	0·2296
Total carbohydrates as hexoses . .	4·1438	7·5728	6·8239	5·0926

The total carbohydrates present in the leaves are less than the quantities present in August, indicating a depressed photosynthetic activity. The cane sugar values are reduced to one half the quantity present in August. In other respects the results of carbohydrate analysis made in September agree with those of August.

TABLE IX.

Results of Sugar analyses of leaves on 18th October 1931.

	6 A.M.	12 Noon	6 P.M.	12 Midnight
Hexoses	0·0286	0·2538	0·2271	0·0306
Cane sugar	5·4604	20·9912	18·6279	6·7724
Starch	0·1248	1·5995	1·9381	0·1194
Total Carbohydrates as hexoses . .	5·6138	22·8445	20·7931	6·9224

The values of the different carbohydrates are higher than the corresponding values in August and September. The value for starch is higher at 6 P.M. than at 12 noon as on former occasions. Another important feature of the results is the highest value for cane sugar at mid-day. This result agrees with that obtained for the mangold leaves by Davis, Daish and Sawyer [1913]. In their results the cane sugar curve reached a maximum at 12 noon.

Measurement of the rate of carbon dioxide absorption

To measure the rate of carbon dioxide absorption by the leaves of the rice plant under constant and uniform conditions of experimentation it was necessary to make use of the continuous current method, first used by Kreusler [1885] and then by a series of workers on photosynthesis. It was necessary, as pointed out by Dastur [1925] to use leaves attached to the plant for making such measurements, and therefore it was undertaken to use the apparatus originally devised by Dastur [1925] with certain modifications suitable for this investigation. A short description of the apparatus is given below.

A big galvanised iron tank is used for keeping the potted rice plant. The pot of the plant was kept under water in the tank (Fig. 4).

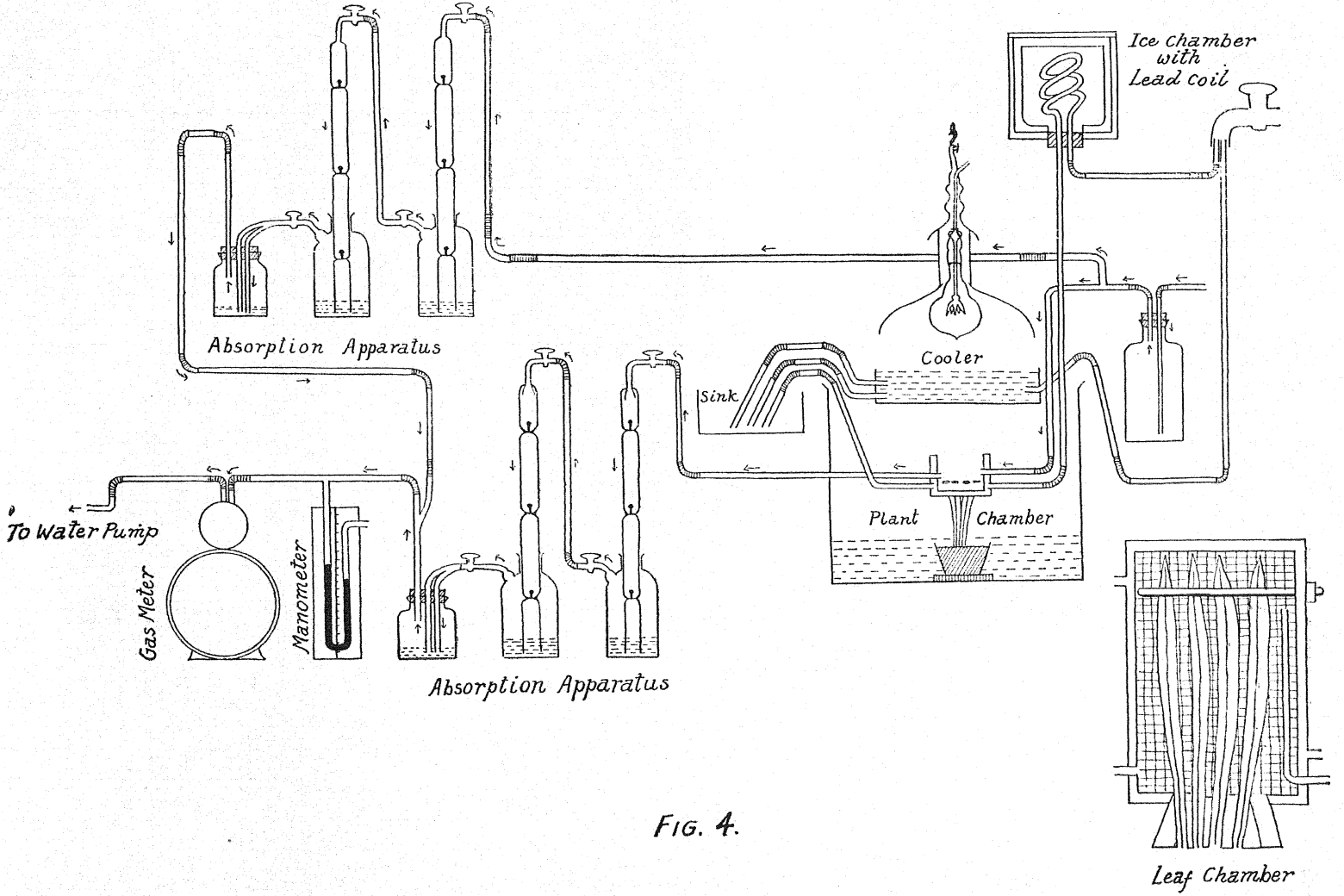


FIG. 4.

Fig. 4.—Apparatus (in cross section) for measuring the carbon dioxide assimilation of the leaves under uniform conditions.

A rectangular box 13 in. by 4 in. by 2—5 in. was made with one of the two larger surfaces made of glass and was used as a leaf chamber. The box has a water jacket half an inch deep surrounding the box on all sides except on the glass surface. There are two holes with projecting tubes made in the water jacket for the inlet and outlet of water. A big rectangular hole 3 in. by $\frac{3}{4}$ in. with a projecting tube $\frac{1}{2}$ in. long is kept for the purpose of inserting four leaves of the rice plant. There are also inlet and outlet tubes for conducting the gas inside and outside of the chamber. There is another small hole made to take a thermometer. A wire gauze is fixed on the floor of the chamber to rest the leaves, so that gaseous exchange on their under surfaces could not be obstructed.

Temperature of the leaf chamber was regulated by circulating the water through the water jacket surrounding the leaf chamber. The flow of the current was regulated so that the temperature of the leaf chamber remained very nearly 30°C., as the temperature of the air in the monsoon months always fluctuated between 28° and 30°C.

Source of light.—Throughout the course of the present investigations, a gas-filled 220 Volt, 1500 Halfwatt Osram, electric bulb was used as a source of light. It was kept at a measured distance of 40 cm. from the leaf. The heat rays emanating from such an electric lamp were cut off by interposing a water cooler in which water was continuously circulated. In this manner all the heat rays were absorbed and the temperature of the leaf chamber remained constant. The layer of water in the cooler was always 2 in. deep in all the experiments. As all the above-named factors are kept constant all throughout, there is no difficulty in obtaining a constant and uniform amount of radiant energy for the leaf per sq. cm. per second in all the cases. This was measured as done by Dastur [1925] by means of a thermopile.

Apparatus for absorbing carbon dioxide.

In all the experiments on the CO_2-assimilation where a current of air or a current of air mixed with a certain percentage of carbon dioxide is passed through the leaf chamber, the results for absorption are obtained by finding out the difference between the carbon dioxide concentration before and after the current passes over the assimilating leaf. The methods followed for the absorption of carbon dioxide by different workers have been different. The determination of the remaining quantity of carbon dioxide is not as difficult in the case of a current having a very high percentage of carbon dioxide, as it is when ordinary air is used. The experimental error is magnified in the latter case, as the quantity of the carbon dioxide present in the air is very small. This necessitates a very accurate arrangement for the absorption of carbon dioxide.

For absorbing carbon dioxide from the air current, Reiset towers are used as done by Kidd, West and Briggs [1921] and by Middleton [1927], as the carbon dioxide is completely absorbed even when the air current is made to flow at a speed of 100 litres per hour. On experimenting with the Reiset towers it was discovered that slight traces of carbon dioxide remain unabsorbed when two Reiset towers were used and therefore it was necessary to put an additional absorbing bottle after the Reiset towers and this devise proved successful. The bottle had two inlet tubes and one outlet tube. The ends of the tubes were drawn so as to produce a fine stream of bubbles. The diameters of the tubes were kept the same. An additional hole in the rubber cork was kept to take the end of burette during the titration operations, so that carbon dioxide of the air may not come in contact with liquid inside the bottle.

As there was a direct air current and also an air current passing through the chamber, two sets of Reiset towers and bottles were used in each experiment. Each set of absorption apparatus consisted of two Reiset towers and one bottle. Pure $\frac{N}{5}$ NaOH (Merck's product) used for absorption was stored in a dark bottle (3 litre capacity) to which a burette with siphon arrangement was attached. By opening a glass stopper the liquid could be made to run into the burette. The end of the burette was fitted in the fourth hole in the stopper of the absorbing bottle and 25 c.c. of the NaOH solution were run into it. 50 c.c. of NaOH solution were run into each Reiset tower. The absorbing apparatus was arranged as shown in Figure 4. The two sets of the absorption apparatus were connected to a manometer which was finally joined to a gas-meter and then to a water pump.

After each experiment the two sets were removed and new ones were replaced.

Gas.—The gas which was drawn through the absorbing sets was ordinary air. It was first of all allowed to pass through a big empty bottle after which the current was bifurcated, one of the bifurcated streams going straight to one set of Reiset towers, the other entering the leaf chamber and through the leaf chamber it entered into the other set of the absorption apparatus.

The idea in intercepting a big empty bottle at the entrance of the gas in the apparatus was to prevent any slight variations in pressure which might be caused by an increase or a decrease in the force of the water running in the pump.

As the total volume of the air drawn through the absorption apparatus during the course of an experiment was nearly 75 to 80 litres, a gas-meter was used to get an idea of the volume of the gas passed through the apparatus.

Experimentation.

The rice plants were grown in pots from the time of transplantation. The seedlings for transplantation were obtained from Karjat for which the authors have to thank the Rice Specialist at Karjat.

It was discovered that rice plant in pots grows best when they (pots) are sunk in the soil in the rice beds. Otherwise the plant does not grow and remains dwarf and in many cases it dies. The rice plants in the sunk pots were as tall and healthy as the plants grown in the rice beds.

For every experiment a potted plant was taken and four leaves most suitable for experimentation were selected.

A mixture of bees wax and paraffin wax (M. P. 50°-52°C.) in equal proportions was used for embedding the leaves in the chamber.

The special feature of the leaf chamber, constructed for the purpose, was the rectangular slit through which the leaves were introduced in it. This arrangement served a double purpose: (1) it facilitated the work of fixing a leaf with a sheathing petiole, (2) it allowed the introduction of more than one leaf into the leaf chamber. The rubber stopper for the slit was prepared from an India rubber slab by cutting and filing it to required proportions. It was cut into two and in one of the halves a slight depression (Fig. 4) was made to rest the leaves on it.

The leaves were carefully fixed between the two halves of the stopper with the mixture of bees wax and paraffin. The leaf chamber was slowly brought near and the leaves were introduced in the chamber through the rectangular slit and melted wax was poured all over the stopper to make the chamber completely air-tight. The leaf chamber together with the potted plant was then lowered into the plant chamber. Great care was taken to see that the leaves were not injured in any way by a pull or by a twist.

The adjustment of the distance of the source of light being made, the light was switched on, the cooler was placed in its proper position and cold water was circulated through it. The outlet tube of the leaf chamber was connected to the water pump and air was drawn for about an hour to allow the leaves to adjust themselves to the external conditions. The flow of water was adjusted in the water jacket surrounding the leaf chamber.

The actual experiment was started generally at 11 A.M. when the air was drawn through the two sets of absorption apparatus for one hour. Three such readings of the assimilation of the carbon dioxide were taken. The light was switched off and the whole apparatus was left in the dark for an hour and the readings for respiration in air deprived of its carbon dioxide were taken.

Estimation.

A number of blank experiments were carried out to test the accuracy of the absorbing apparatus. The current of air introduced into the apparatus was bifurcated, one of the streams going direct to one set and the other to the second set

of the absorption apparatus after passing through the leaf chamber. The following experiments were performed to prove the efficacy of the absorbing apparatus.

A current of air was drawn for an hour through the two sets of absorption apparatus, each Reiset tower and bottle containing 50 c. c. and 25 c. c. of $\frac{N}{5}$ NaOH solution respectively. All the connections were made as described before with the difference that no leaves were enclosed in the leaf chamber during the performance of these experiments. The following are some of the results obtained when the rate of bubbling is 20 bubbles per 30 seconds.

TABLE X.

The amount of carbon dioxide absorbed by the two absorption sets from the air streams, 21st June 1930.

Chamber stream	Direct stream	
	Grms.	Grms.
50 c. c. NaOH, 1st Reiset tower.	0·010408	0·010630
50 c. c. „ 2nd „	0·009080	0·008192
25 c. c. „ 1st bottle	0·001108	0·001772
Total absorption in each set . .	0·020596	0·020594

The values given in Table X above are the amounts of carbon dioxide in grms. absorbed individually by each Reiset tower and bottle. It becomes evident that the air current is exactly halved. The total absorption of carbon dioxide in each set of the absorption apparatus is the same.

To ascertain whether the same volume of gas is passed through the apparatus every time, readings were taken at different times during the day and also on different days. On comparing the results of absorption of CO_2 taken on 21st June 1930 (given in the preceding table) and those taken on 28th June 1930, which are tabulated below, it will be seen that the volume of the air current passed through the apparatus remains fairly constant in all the experiments.

TABLE XI.

Rate of bubbling—20 bubbles per 30 seconds.

Chamber stream	Direct stream	
	Grms.	Grms.
50 c. c. NaOH 1st Reiset tower	0·010408	0·010630
50 c. c. „ 2nd „ „	0·009078	0·009080
25 c. c. „ 1st bottle	0·001772	0·001550
Total absorption in each set . .	0·021258	0·021260

It was found that the rate of 20 bubbles per 30 seconds gave higher values for the carbon dioxide absorption than when the air was bubbled through the absorption apparatus at a greater speed.

Hydrochloric acid solution.—Four c. c. of concentrated hydrochloric acid were diluted with a litre of distilled water and its strength determined in the following manner:—

0·6N succinic acid solution was prepared and 10 c. c. of it were titrated against NaOH solution from the siphon burette. 25 c. c. of the dilute HCl were then titrated against the standardised NaOH solution. The indicator used was the Universal Indicator supplied by the British Drug House.

Sodium hydroxide solution.—NaOH solution $\left(\frac{N}{5}\right)$ for absorption was prepared by dissolving a weighed quantity of pure NaOH sticks in a litre of water. The volume was made to 3 litres in the bottle attached to the siphon-burette. The NaOH sticks contain a certain amount of initial carbonate which must be determined and the amount should be deducted from the figure obtained for the absorption of CO_2 to realise the true value of absorption.

Titration.—The NaOH solution was titrated with standard HCl solution using the Universal Indicator; 4 or 5 drops of Universal Indicator were put in the Reiset tower or the bottle containing the NaOH solution and violet colouration was given to the solution. This was titrated with about 0·2N HCl. This acid neutralised the NaOH and half the bicarbonate. The end-point of the reaction was shown by greenish yellow colour. The solution was finally titrated with standard HCl (0·05 N) to determine the other half bicarbonate, the end-point being marked by pink colouration. The total carbonate is obtained by multiplying the figure obtained by 2. Thus the carbonate present after the absorption is determined. The initial carbonate present before the absorption is subtracted to get the real value of the carbonate after the air current is drawn through the solution. Similar determinations are carried out for every Reiset tower and bottle. The total carbonate value of the stream passing through the leaf chamber is subtracted from the total carbonate value of the direct stream, to obtain the value for apparent assimilation. From the data, assimilation per unit area could be calculated out.

The respiratory values are also obtained in the same manner and they are added to the corresponding values for apparent assimilation. The leaves absorb certain amount of carbon dioxide from the air current which is given by the difference of the absorption of the direct and leaf chamber streams plus the carbon dioxide evolved in respiration during the period of the experiment; thus if a represents the difference in the absorption values of direct and leaf chamber

streams and b the amount of carbon dioxide evolved in respiration during the period of the experiment. $a + b$ will be the real assimilation.

TABLE XII.

Apparent assimilation of CO_2 in grms. per 100 sq. cm. per hour.

Date	Reading No. 1, 11 a.m. to 12 noon	Reading No. 2, 12-30 to 1-30 p.m.	Reading No. 3, 2 to 3 p.m.
22nd July 1930 . . .	0·00385 grms.	0·00412 grms.	0·003573 grms.
1st August ,, . . .	0·00569 ,,	0·00510 ,,	0·00569 ,,
5th ,, ,, . . .	0·000765 ,,	0·00790 ,,	0·00814 ,,
10th ,, ,, . . .	0·00673 ,,	0·00621 ,,	0·00655 ,,
15th ,, ,, . . .	0·00678 ,,	0·00678 ,,	0·00717 ,,
18th ,, ,, . . .	0·00702 ,,	0·00735 ,,	0·00736 ,,
21st ,, ,, . . .	0·00726 ,,	0·00759 ,,	0·00743 ,,
25th ,, ,, . . .	0·00725 ,,	0·00768 ,,	0·00768 ,,
30th ,, ,, . . .	0·00776 ,,	0·00746 ,,	0·00761 ,,
5th September 1930 . .	0·00761 ,,	0·00763 ,,	0·00770 ,,
11th ,, ,, . .	0·00701 ,,	0·00708 ,,	0·00704 ,,
15th ,, ,, . .	0·00714 ,,	0·00752 ,,	0·00733 ,,
19th ,, ,, . .	0·00734 ,,	0·00771 ,,	0·00752 ,,
23rd ,, ,, . .	0·00734 ,,	0·00751 ,,	0·00768 ,,
28th ,, ,, . .	0·00752 ,,	0·00781 ,,	0·00796 ,,
3rd October ,, . .	0·00973 ,,	0·01020 ,,	0·01081 ,,
7th ,, ,, . .	0·01730 ,,	0·01758 ,,	0·01786 ,,
8th ,, ,, . .	0·01556 ,,	0·01580 ,,	0·01603 ,,
10th ,, ,, . .	0·01650 ,,	0·01700 ,,	0·01690 ,,
11th ,, ,, . .	0·01530 ,,	0·01552 ,,	0·01508 ,,
15th ,, ,, . .	0·01603 ,,	0·01650 ,,	.....
16th ,, ,, . .	0·01512 ,,	0·01664 ,,	0·01621 ,,
17th ,, ,, . .	0·01355 ,,	0·01403 ,,	0·01308 ,,
31st ,, ,, . .	0·00748 ,,	0·00765 ,,	0·00732 ,,

TABLE XIII.

Readings for respiration amount of CO_2 in grms. per 100 sq. cm. given out by respiration in one hour.

Date	Reading No. 1, 4 to 5 p.m.	Reading No. 2, 5-30 to 6-30 p. m.
22nd July 1930	0·00659 grms.	....
1st August ,, ...	0·00549 ,,	0·00588 grms.
5th ,, ,, ...	0.00839 ,,	0·00888 ,,
10th ,, ,, ...	0·00947 ,,	0·00947 ,,
15th ,, ,, ...	0·00988 ,,	0·00949 ,,
18th ,, ,, ...	0·00902 ,,	0·00969 ,,
21st ,, ,, ...	0·00969 ,,	0·00985 ,,
25th ,, ,, ...	0·01015 ,,	0·01000 ,,
30th ,, ,, ...	0·01010 ,,	0·01005 ,,
5th September 1930	0·00952 ,,	0·00928 ,,
11th ,, ,, ...	0·01009 ,,	0·01000 ,,
15th ,, ,, ,, ...	0·00921 ,,	0·00930 ,,
19th ,, ,, ...	0·00905 ,,	0·00901 ,,
23rd ,, ,, ...	0·00973 ,,	0·00939 ,,
28th ,, ,, ...	0·008696 ,,	0·00899 ,,
3rd October ,, ...	0·01019 ,,	0·01030 ,,
7th ,, ,, ...	0·01326 ,,	0·01211 ,,
8th ,, ,, ...	0·01109 ,,	0·01155 ,,
10th ,, ,, ...	0·01462 ,,	0·01486 ,,
11th ,, ,, ...	0·01419 ,,	0·01441 ,,
15th ,, ,, ...	0·01369 ,,	0·01415 ,,
16th ,, ,, ...	0·01405 ,,	0·01405 ,,
17th ,, ,, ...	0·01169 ,,	0·01215 ,,
31st ,, ,, ...	0·00830 ,,	0·00845 ,,

TABLE XIV.

"Real" CO_2—assimilation.

Date	Total amount of CO_2 absorbed in grms. per 100 sq. cms. of leaf area	Date	Total amount of CO_2 absorbed in grms. per 100 sq. cms. of leaf area
1930.		*1930.*	
22nd July . .	0·01023 grms.	19th September .	0·01652 grms.
1st August . .	0·01120 ,,	23rd ,, . .	0·01706 ,,
5th ,, . .	0·01654 ,,	28th ,, . .	9·01720 ,,
10th ,, . .	0·01610 ,,	3rd October . .	0·02043 ,,
15th ,, . .	0·01651 ,,	7th ,, . .	0·03026 ,,
18th ,, . .	0·01660 ,,	8th ,, . .	0·02711 ,,
21st ,, . .	0·01720 ,,	10th ,, . .	0·03103 ,,
25th ,, . .	0·01754 ,,	11th ,, . .	0·02961 ,,
30th ,, . .	0·01761 ,,	15th ,, . .	0·03018 ,,
5th September .	0·01714 ,,	16th ,, . .	0·03004 ,,
11th ,, . .	0·01711 ,,	17th ,, . .	0·02543 ,,
15th ,, . .	0·01654 ,,	31st ,, . .	0·01588 ,,

Table XII gives the values for apparent assimilation of CO_2 per sq. decimeter of leaf area per hour from 11 A.M. to 3 P.M. These determinations were made from the 22nd July up to 31st October. The values of CO_2-assimilation obtained vary slightly from hour to hour and it may be due to variations in the photosynthetic activity of the leaves. Such diurnal variations of the photosynthetic activity with low concentrations of carbon dioxide at constant intensity of light have been recorded by Maskell [1928]. The rate of apparent assimilation in the early stages of growth is about 3 to 6 milligrams per sq. decimeter of leaf-surface per hour, and then the rate of apparent assimilation remains fairly uniform during the months of August and September. Highest rate of apparent assimilation is found in October when the value goes as high as 16 milligrams per 1 sq. decimeter of the leaf-surface per hour. Towards the close of October the rate of assimlation falls rapidly.

Table XIII gives the values for respiration. Two determinations of respiration are made every time when the apparent CO_2- assimillation is measured. The amount of CO_2 respired is generally higher than the amount of CO_2 absorbed, as

comparison of the figures in Tables XII and XIII will show. The respiratory activity is highest when the apparent assimilation is highest.

Table XIV gives the values of true or "real" assimilation obtained by adding the mean value of the three apparent assimilation values to the mean value of the two respiration readings. The real assimilation for the leaves of the rice plant fluctuates between 10 and 30 mgrms. per 100 sq. cm. (or 1 sq. decimeter), of the leaf-surface per hour.

The rate of assimilation remains fairly constant in the month of August and shows a slight rise and towards the end of August and in the beginning of September the rate of assimilation reaches a higher value on the 19th of September after which there is a fall. The second big rise in assimilation occurs in the first week of October, *i.e.*, at the flowering stage. From the 10th October to the 16th October the rate of assimilation is highest and then there is a decline.

The graph showing the real assimilation at different stages of growth is shown in Fig. 5. The great rise in the rate of assimilation in October, *i.e.*, between 88 and 98 days after transplantation is clear from the graphs.

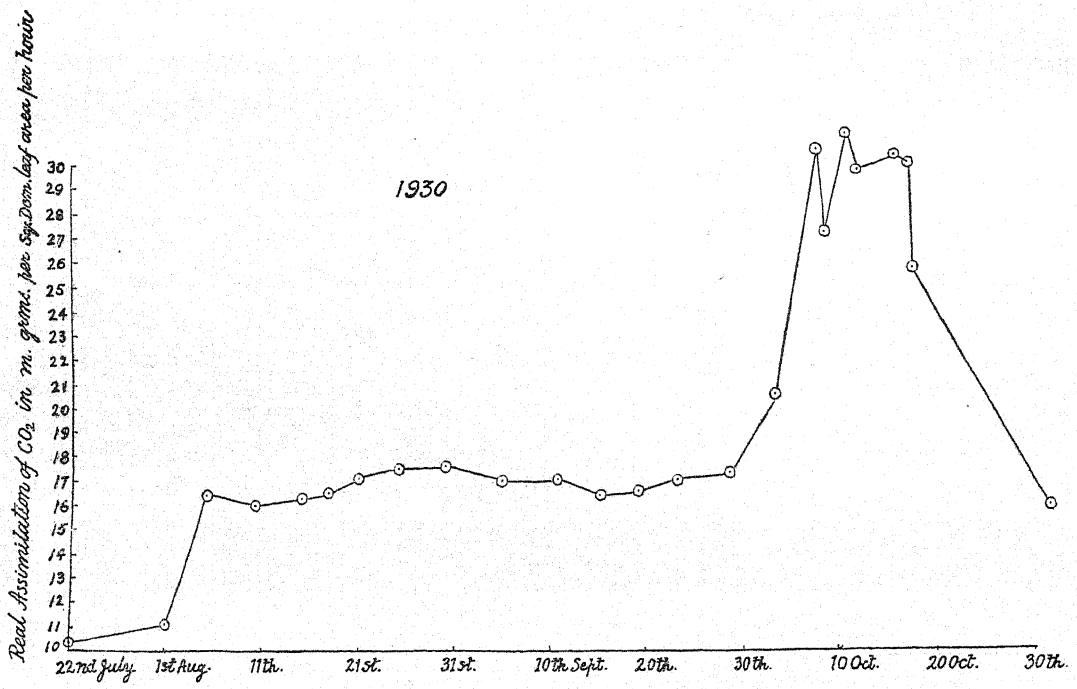


Fig. 5.—Real assimilation of carbon dioxide in milli-grams per hour per 1 sq. deci-meter of the leaf area.

Conclusions.

As the photosynthetic activity of the rice plant is determined by two independent methods, it would be of interest to compare the results thus obtained. The values of the photosynthetic activity of the rice plant obtained either as total hexoses formed or as carbon dioxide assimilated could not be compared by converting hexoses into carbon dioxide or *vice versa*. In one case photosynthesis is measured in sunlight and in the other case in artificial light, and as shown by Dastur and Samant the lights from the two sources have not the same effects on photosynthesis. But the general trend of photosynthetic activity during the season as determined by these two methods can be compared as, though the actual values obtained may differ in the two cases, the rise and fall in the values should occur concurrently in both measurements. When the graphs illustrating the photosynthetic activity determined by the carbohydrate analysis method (Figs. 1 and 3) are compared with the graph (Fig. 5) illustrating the photosynthetic activity determined by the carbon dioxide absorption method, it will be noticed that they both show the same characteristic features, that is, the rise and fall in the curves coincide and the general nature of the curves is the same in two cases.

This investigation has conclusively shown that the photosynthetic curve has two maxima, one in August and the other in October, the second being much higher than the first.

Summary.

As no data is collected, so far as the authors are aware, about the photosynthetic activity of the leaves of the rice plant by direct measurements, it is undertaken to measure the rate of photosynthesis of the leaves of the rice plant at different stages of growth.

The photosynthetic activity of the leaves in this investigation is measured in two ways (1) by determining the carbohydrate contents of the leaves in the morning and in the evening, and (2) by measuring the absorption of carbon dioxide from the air under controlled and uniform conditions of experiments.

In the first method the leaves are killed and extracted with alcohol taking necessary precautions. The sugars are then separated from other substances. The starch is hydrolysed by taka-diastase and is converted into dextrose and maltose.

All sugars are estimated as hexoses. Cane sugar and maltose are hydrolysed to hexoses before estimations. A colorimetric method of estimating sugars first used by Folin and Wu [1918] and subsequently modified by other workers is used in this investigation with further modifications.

A sample of leaves is taken in the morning and is analysed for carbohydrates. Another sample is taken the same evening and is similarly analysed. Twenty such

double carbohydrate analyses of the leaves are made on different days from July to October. On two occasions the leaves are analysed for carbohydrates every six hours during 24 hours.

In the second method carbon dioxide absorption of the leaves of the rice plant is measured by the continuous gas current method. A special leaf chamber to hold four leaves of the rice plant is devised. Ordinary air is used and is circulated through the leaf chamber at the rate of about 100 litres per hour. The carbon dioxide of the air before and after it passed through the leaf chamber is estimated in two sets of absorption apparatus consisting of Reiset towers containing NaOH solution of known strength.

From the results obtained by the two methods the following periodicity in the photosynthetic activity of the leaves of the rice plant is noticed. The photosynthetic activity rises rapidly soon after transplantation. It remains fairly uniform in August after which there is a depression in September. There is a sudden rise in the photosynthetic activity in October and it reaches its second maximum during that period. There is a big fall in the rate of photosynthesis soon after.

The same features of the photosynthetic activity are noticed in the results obtained by the two methods.

REFERENCES.

Brown, H. T. and Morris, G. H. (1893). *J. Chem. Soc. Trans.* **63**, 604.
Calvert, E. G. B. (1923). *Bio-Chem. J.* **17**, 117.
——— (1924). *Bio-Chem. J.* **18**, 839.
Dastur, R. H. (1925). *Ann. of Bot.* 779.
Dastur, R. H. and Samant, K. M. In course of publication in the *Annals of Botany.*
Davis, A., Daish, A. J. & Sawyer, A. J., A. M. (1913). *J. Agric. Sci.* **5**, 437.
Folin and Wu (1918). *J. Biol. Chem.* **38**, 106.
Gile, P. L. and Carrero, J. O. (1915). *J. Agric. Res.* **3**, 205-210.
Kreusler, M. (1885). *Landw. Jahrb.* **14**, 913-965.
Kidd, F., West, C. & Briggs, G. E. (1921). Proc. Roy. Soc. Lond. B. **92**, 368.
Kelley; W; P. & Thompson, A. R. (1910). *Hawaii Agric. Exp. Stat. Bull.* No. **21**.
Kelley, W. P. (1911). *Hawaii Agric. Exp. Stat. Bull.* No. 24.
Lewis, F. J. & Tuttle, G. M. (1920). *Ann. of Bot.* **34**, 405.
Maskell, E. J. (1928). Proc. Roy. Soc. (London) B. **102**, 467, 488.
Middleton, N, J. (1927). *Ann. of Bot.* **41**, 345.
Miyake, K. (1914). *J. Coll. Agric. Tohoku Imp. Univ. Sapporo.* **52**, 241.
Parkin, J. (1911). *Bio-Chem. J.* **6**, 1.
Sahasrabuddhe, D. L. (1928). *Dept. Agric. Bomb. Bull.* No. 154.
Sen, J. N. (1916). *Agric. Res. Inst., Pusa., Bull.* No. 65.

NITROGEN RECUPERATION IN THE SOILS OF THE BOMBAY PRESIDENCY, PART III.

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(With 7 text-figs.)

I.—Introduction.

It is a well known fact that of all the plant food ingredients in the soil, nitrogen is by far the most important, especially in the arid and semi-arid tracts of the Bombay Deccan. In these tracts crops are grown year after year, on rains, without any serious attempt to return to the land the plant food ingredients removed by crops. A question therefore naturally arises as to whether the soils are able to recuperate the nitrogen by directly fixing it from the air, to make up for the loss they sustain due to growing of crops, and if so, how far this fixation is affected by the nature of the soil and by the weather conditions to which the soils are exposed. Investigation in this direction was taken up as far back as 1923 and the first results were published in 1925 as a Memoir with the title "Nitrogen Recuperation in the Soils of the Bombay Deccan, Part I" [Sahasrabuddhe and Daji, 1925]. The experiments were conducted in the laboratory on the medium black soil of the Deccan, taken at the end of the hot weather, kept dry and then exposed to varying conditions. The results showed that a good deal of nitrogen fixation takes place in the trap soils when they are moistened and kept at suitable temperatures. Further work on the subject was published in this *Journal* [Sahasrabuddhe and Ghatikar, 1931]. For the investigation published in this paper, typical soils of the Bombay Presidency were obtained and the effects of the additions of lime, phosphatic substances, organic matter and alkali salts to these soils were studied. The results definitely prove that, at least under the controlled conditions of the laboratory, the additions of phosphatic substances and organic matter improve the nitrogen recuperation of such soils as are not originally rich in these ingredients.

But it remained to be seen whether any recuperation of nitrogen takes place actually under field conditions where there is repeated wetting and drying of soils

by rain and heat. It was therefore necessary to determine whether the fixation of nitrogen takes place under field conditions, just as it does in the laboratory. Such studies could only be undertaken if plots in fields could be regularly kept under observation and where data with regard to the previous history and the present conditions of cultivation and crops could be obtained. Such a suitable place was found at the Dry Farm Experiment Station at Manjri, about 8 miles to the east of Poona. Here all the necessary information together with the record about the meteorological factors was available.

The Dry Farm Experiment Station was opened early in 1924 on what may be called a virgin land previously occupied by scrub jungle and *babul* (*Acacia arabica*) shrubs. The area was divided into suitable plots of 1/40th of an acre or one *guntha*. Dry farming experiments are being conducted on these plots since then. Majority of the experiments are of cultural nature and do not involve any addition of manures. From 1924 to 1930 seven crops of *jowar* (*Andropogon Sorghum*) were raised during the *rabi* season from September to February. The yields obtained were much larger than the average yields of farmers and it was feared that these annual harvests of heavier yields might result in depleting the soil of its fertility. Under the dry arid conditions of the Deccan nitrogen is liable to far greater losses than any other plant food ingredients, and from 1927 onwards nitrogen determinations were made every year sometime after the harvest of the crop. The following figures denoting nitrogen contents of the soil from year to year would be found interesting. The samples were taken from an area measuring 12 *gunthas* or 3/10th of an acre. Column 2 in the following table gives nitrogen from this plot which received no manure during the period of experiments, while column 3 gives nitrogen from a plot which received farmyard manure. *Rabi jowar* was grown annually from September to February on both the plots.

TABLE I.

Percentages of total nitrogen in oven-dry soil.

Date of taking sample	Nitrogen from soil receiving no manure	Nitrogen from soil receiving farmyard manure
	Per cent.	Per cent.
30th September 1924	0·120	0·120
3rd February 1927	0·099	0·097
2nd February 1928	0·085	0·079
22nd February 1929	0·081	0·096
14th April 1930	0·079	0·075

The figures in column 2 by themselves indicate an alarming situation in the rapid depletion of soil fertility. But the above decrease in nitrogen contents of the

soil was not found to have resulted in lowering of yields from these plots. Further, there is a similar depletion of nitrogen from a plot which received farmyard manure. The quantity of farmyard manure was as large as 10,000 lbs. per acre of total manure given in 1924, 1925, 1926, 1927 and 1929.

In both the series of plots the annual yields do not account for the heavy losses of nitrogen during the period of experiments as indicated by figures given on another page (page 459).

This general downward trend of nitrogen contents in all the plots irrespective of their treatments was contrary to expectations. The experiments in the laboratory showed that a definite recuperation of nitrogen takes place in the medium black soils of the Bombay Deccan when wetted and kept at suitable temperatures. Under the natural climatic conditions obtained at Manjri there is wetting and heating of soils. But the above results indicated apparently a decided depletion of soil nitrogen from year to year during a period of six years.

During the crop season of 1928-29 nitrogen determinations were made twice from the same plots once before the advent of the monsoon rains and again after heavy rains of September. And it was observed that the plots which had shown the lowest nitrogen content before the rains showed a comparatively high nitrogen content after the rains. Further determinations after the crop harvest again showed a lowering of soil nitrogen. These duplicate plots had received treatment of having furrows and ridges made.

TABLE II.

Percentage of nitrogen at different seasons in plots having ridges and furrows.

Date of sampling	Percentage of nitrogen
February 1928	·057
September 1928	·080
February 1929	·061

This rise in nitrogen in plots to which no manure was ever added as opposed to the general trend of lowering of nitrogen contents gave a distinct indication of the nitrogen recuperation under field conditions and showed that the time of taking a soil sample has some influence on nitrogen contents of the soil. This means the effect of the climatic conditions of heat and rain and the subsequent moisture contents of the soil must be taken into account.

It was therefore decided to carry on a series of determinations of the total nitrogen contents of soils receiving different treatments of cultivation and manuring throughout one complete year taking the soil samples once every month.*

* The authors' thanks are due to Mr. D. M. Ranade of the Chemical Laboratory and to Mr. G. M. Bapat of the Soil Physicist Section, for the help they rendered in the experimental work.

It would be seen from the results that follow that the nitrogen content of soils is not a constant quantity. It is liable to fluctuations from season to season. The recuperation of nitrogen seems to take place under the natural conditions of atmospheric influences as definitely as under the controlled conditions in the laboratory. It would be seen in the following account that the most active period of nitrogen recuperation corresponds with the most active period of crop growth especially when the growing crop is the winter or *rabi* crop. The impression of rapid depletion of soil nitrogen from the figures of soil nitrogen obtained from year to year without any attention to time of sampling or the moisture condition of the soils must be modified in the light of studies described here.

II. Recuperation of nitrogen in unmanured soils.

The piece of land which was selected for the study of the nitrogen contents of the soil for a period of twelve months consisted of a rectangular block measuring 3/10th of an acre divided into 12 small equal plots. The length of the rectangular block was 132 feet and the width 99 feet. Except in the cultivator's plots described later precautions were taken to stop any run-off of rain water by placing small bunds 9 in. high all round. Hence there was no possibility of loss of soil material or soil nitrogen by surface erosion.

Before the land was put under any crop, the usual chemical and physical analyses were done and the soil depths of plots were determined by auger tests or where necessary by digging pits. Levels were taken to find out the slope in any direction.

The general morphological study as well as the analytical data indicate that the soil is the medium black soil. It has *murum* as a sub-soil layer in most cases, while on slopes the sub-soil layer is a mixture of lime infiltrations.

Table III.

Chemical analysis of soil from surface layer of 6 in. depth, sample being taken on the 30th September 1924.

	Percentages
Total moisture	22·30
Stones	2·20
	On fine ovenn-dry matter
Loss on ignition	7·75
Insoluble matter (silica)	60·71
Alumina and iron oxide	14·12
Lime as CaO	9·64
Phosphoric acid (P_2O_5)	0·073
Potash as (K_2O)	0·39
Nitrogen	0·120
Organic matter	2·26

The chemical analysis indicates that the soil on account of its virgin nature is very rich in nitrogen and organic matter. Potash is present in sufficient amount while the lime contents are above the average. The quantity of phosphoric acid, though not high, may be looked upon as fair.

The mechanical condition indicates that the soil belongs to the clay loam type.

This piece of land is under cultivation ever since the land was opened by the plough for the first time early in 1924. From 1924 to 1930 no manure of any kind was ever added and the only crop grown was the *rabi jowar* (*Andropogon Sorghum*) year after year with the usual tillage.

From the results of volume weight it is computed that the acre-foot layer of soil would be equal to nearly four million pounds in weight. The quantity of total nitrogen in the three quarters of a foot of soil from one acre on 30th September 1924 amounted to 3,600 lbs., while on the 14th April 1930 it was 2,370 lbs. thus showing a loss of 1,230 lbs.

The annual harvesting of *jowar* crop cannot account for this enormous loss in soil nitrogen. The record of the dry matter of the harvested crops shows the quantities of nitrogen used up by the crop in different years as follows :—

TABLE IV.

Yields of jowar *crop in lbs. including grain and stalks and pounds of nitrogen removed in different years.*

Year	Yield per acre	Nitrogen removed per acre by the crop
	lbs.	lbs.
1924	4,217	42
1925	2,148	21
1926	2,467	24
1927	4,619	46
1928	3,423	34
1929	2,541	25
1930	2,534	25
		217

The chemical analysis of the matured plant of *jowar* gives the nitrogen content as 1 per cent. Hence the quantities of nitrogen removed by the crop from year to year would be represented as above in round numbers. Thus the total quantity of nitrogen removed by the crop grown on the land during the period of seven years amounts only to 217 pounds.

It is obvious therefore that the enormous apparent decrease of 1,230 lbs. of nitrogen of the soil from 1924 to 1930 cannot be accounted for by the removal of nitrogen by the crops raised. The question therefore arises whether the depletion of soil nitrogen in seven years is real or only apparent. In order to get a definite and satisfactory answer a careful study of the nitrogen contents of the soil for a period of one complete year was taken up by examining the nitrogen contents once every month. A careful record of all operations done during the period as also the moisture contents and temperatures of the soil was kept for correlating them to the nitrogen contents.

Method of soil sampling.—It has been already shown that the rectangular area of 3/10th acre from which determinations of nitrogen were regularly made is divided into 12 equal plots for experimental purposes. The sampling was done by means of a screw auger, taking the samples to a depth of 9 in. from the surface. One sample was taken from every plot in this way and all the twelve samples were then mixed together to obtain a composite sample. This sample was immediately brought to the laboratory and rapidly dried on a water bath at a temperature of 60°C. Simultaneously moisture determination was made in the water oven at 98°C. The quickly dried sample at 60°C. on water bath was then brought to Poona to the laboratory of the Agricultural Chemist for the determination of the total nitrogen as well as nitrogen in other forms. It may be pointed out that in the first special trials that were made the average of twelve separate nitrogen determinations gave exactly the same figure as was obtained for the composite sample.

The usual Kjeldahl's method was used for determination of the organic and ammoniacal nitrogen together, while the nitrate and the nitrite nitrogens were determined by the phenoldisulphonic acid method and the Gress Ilosway method respectively, as described in Appendix of Part I. The determinations of nitrogen contents were commenced in July 1930 and continued till June 1931, for twelve months so as to complete a cycle of all the seasons of the year. Prior to commencing the above determinations the following preparatory tillage was done to the land.

The land was ploughed on 17th March 1930 and then harrowed three times before the sample of soil was taken on the 18th July for the first determination of nitrogen of the series. The first harrowing was given on the 5th of May, the second

on the 25th May and the third on the 28th of June 1930. The tabular statement given below would indicate the various operations done to the land.

TABLE V.

Date	Nature of operation
28th July 1930	Harrowing with tooth-cultivator
17th September 1930	Sowing of *jowar* crop and covering
1st October 1930	Stirring the upper surface by hand-hoe
16th October 1930	" " " "
11th November 1930	" " " "
2nd December 1930	" " " "
29th December 1930	" " " "
14th February 1931	Harvesting of *jowar* crop by up-rooting
25th March 1931	Ploughing of the land with a CT_2 plough
23rd April 1931	Harrowing with a blade harrow
24th May 1931	" " " "

The above data are important as the stirring of the soil naturally changes the moisture content and exposes the loosened particles to the atmospheric agencies which affect the nitrogen contents. Again the climatic factors like the rainfall and temperature are also responsible in bringing about changes in the nitrogen contents and hence these have been given below in detail before the actual nitrogen contents of the soil are discussed.

The rainfall at the Dry Farm Experiment Station from June 1930 to June 1931 is given below for every fortnight.

TABLE VI.

Fortnightly and monthly rainfall in inches during the period of experiment.

——	June 1930	July 1930	Aug. 1930	Sep. 1930	Oct. 1930	Nov. 1930	Dec. 1930	Jan. 1931	Feb. 1931	Mar. 1931	April 1931	May 1931	June 1931
1st fortnight	0·40	1·92	0·75	4·66	3·16	3·46	0·05	..	..	0·00	..	..	0·31
2nd fortnight	1·45	0·92	1·20	0·62	1·22	..	..	..	..	0·28	..	..	0·96
Total during the month	1·85	2·84	1·95	5·28	4·38	3·46	0·05	..	..	0·28	..	..	1·27

The following table gives the average maximum air temperature of each month along with the soil temperatures.

TABLE VII.

Average maximum temperature of the air during each month and the average soil temperature at two different depths all expressed in centigrade degrees.*

——	June 1930	July 1930	Aug. 1930	Sep. 1930	Oct. 1930	Nov. 1930	Dec. 1930	Jan. 1931	Feb. 1931	Mar. 1931	April 1931	May 1931	June 1931
Average maximum air temperature .	34·5	26·5	25·6	27·0	30·1	25·9	28·5	26·6	31·7	32·2	38·4	36·5	33·0
At a depth 3 in. from surface .	31·5	27·5	28·7	27·5	29·7	27·9	30·4	2·1	33·6	36·6	41·3	41·5	..
At a depth 6 in. from surface .	31·1	27·2	28·6	27·4	29·4	27·7	28·6	28·7	30·2	32·2	37·3	39·1	35·3

It has already been stated that the nitrogen content of the soil on the 14th April 1930 was found to be only 0·079 per cent. or 79 milligrams in 100 grams of oven-dry soil. The regular monthly determinations were commenced in July 1930 as already stated above. The first sample was taken on the 18th July 1930. The subsequent samples were taken on the same date as far as possible in the following months, change being made when climatic conditions made it necessary to postpone the same. The following table shows the total nitrogen contents of the soil along with the date of taking the sample. This figure has been arrived at by adding the nitrogen obtained in the form of nitrates and nitrites to the nitrogen obtained by the Kjeldahl process which gives the organic and ammoniacal nitrogen. Taking the nitrogen in July 1930 as 100, the percentage variation from this is expressed in the last column.

TABLE VIII.

Milligrams of total nitrogen in soil from month to month on 100 grams of oven-dry soil.

Date of sampling	Total nitrogen in milligrams per 100 grams of soil	Variation in per cent. over the original
18th July 1930	73·61	100·0
18th August 1930	98·12	133·3
25th September 1930	80·95	109·9
18th October 1930	99·38	134·9

* The soil temperatures given were recorded daily at 5-30 P.M. and the monthly averages were calculated from them. It may be pointed out that the average soil temperature at 3 in. depth at this time is slightly higher than the maximum air temperature of the day while the temperature at a lower depth at 6 in. is slightly below the maximum temperature of air.

TABLE VIII—*contd.*

Milligrams of total nitrogen in soil from month to month on 100 grams of oven-dry soil—contd.

Date of sampling	Total nitrogen in milligrams per 100 grams of soil	Variation in per cent. over the original
18th November 1930	102·13	138·7
18th December 1930	121·62	165·1
18th January 1931	118·31	160·6
20th February 1931	118·30	160·6
23rd March 1931	108·70	147·6
21st April 1931	107·87	146·4
20th May 1931	100·63	136·7
21st June 1931	105·81	143·6

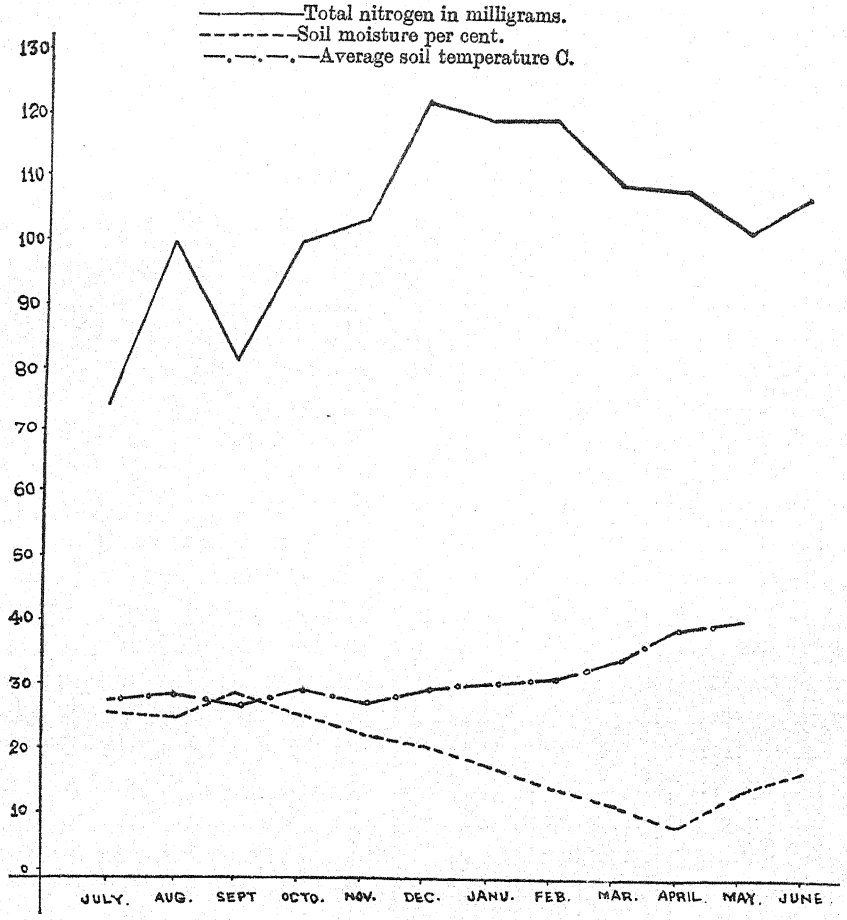


Fig. 1.

It is interesting to trace the changes in nitrogen content of this series in comparison with the actual soil temperatures observed together with the moisture contents of the soil from month to month as given in the table below and illustrated by Fig. 1.

TABLE IX.

Total nitrogen in 9 in. surface soil, together with the corresponding soil moisture and average monthly soil temperature.

Month	Total nitrogen, milligrams in 100 grams of oven-dry soil	Per cent. moisture	Average monthly soil temperatures—average of temperature at 3 in. and 6 in. depth from surface
July 1930	73·6	25·6	27·3
August 1930	98·1	24·1	28·6
September 1930	80·9	28·1	27·4
October ,,	99·4	25·9	29·5
November ,,	102·1	22·3	27·8
December ,,	121·6	20·2	29·5
January 1931	118·3	17·7	30·4
February ,,	118·3	14·4	31·9
March ,,	108·7	11·3	34·4
April ,,	107·9	8·0	39·3
May ,,	100·6	13·6	40·3
June ,,	105·8	16·3	—

When the determinations were commenced after the middle of July, the monsoon had just set in and had moistened the upper six in. layer of the surface soil. The soil temperatures which were high going up to 40°C., had come down giving an average soil temperature of 27·5°C. The nitrogen content of the soil was then at its minimum being 73 milligrams per hundred grams of the soil. The effect of wetting by the monsoon rains together with a slight rise in soil temperature was to increase the nitrogen to 98 milligrams or a rise of 33 per cent. in a month's period after the original determination. The heavier rain in the month of September amounting to 5·75 inches, combined with a fall in temperature by over a degree, resulted in lowering the nitrogen content to 80 milligrams or a fall of 17 per cent. over the previous determination. The next determination again showed an increase in nitrogen to the extent of 34 per cent. over the original. There was a little more than 3 in. of rain during this period but the average temperature had increased by over 2°C. In the following month there was a slight rise in nitrogen but the peak of the curve reached its highest in December. There was practically no rain between the determinations of November and December and the average soil temperature was higher than even before since the commencement of the experiment. The maximum rise in nitrogen contents was as much as 65 per cent. over

the original. During the next two months the nitrogen remained fairly high under the high temperatures of January and February. Then a steady fall in nitrogen continued throughout the following four months from March to June with steady lowering of the moisture contents of the soil, though there was a steady rise in temperature. At the end of twelve months' period of experiment the soil was left richer in its nitrogen contents than at its commencement being nearly 43 per cent. higher. The various operations that were carried during the period of the experiment have been already given above. The land was occupied by a crop of *rabi jowar* from the 17th of September 1930 to the 14th of February 1931. In spite of the presence of the crop on the land, it was freely exposed to the sun, rain and wind, as the plant population on the experimental plots was very limited. The crop was sown by dibbling 2 seeds 18 in. each way. The roots and leaves of plants if obtained in the soil sample were carefully picked up and removed so as to avoid any addition of nitrogen due to their inclusion.

Under natural field conditions definite recuperation of nitrogen seems to start on the advent of the monsoon rains. Again this recuperation is much more pronounced and continues for a much longer period than is found under laboratory conditions. The maximum fixation of nitrogen was obtained when the average maximum soil temperature was about 29·5°C. and when the moisture content was about 20 per cent. The combined effect of moisture content and temperature in December resulted in fixing far larger quantities of nitrogen than are obtained under laboratory conditions. Greaves [1918] mentions 20 per cent. moisture as the optimum for nitrogen fixation found by Warmbold and also quotes 28°C. as the optimum temperature. The optimum temperature for nitrogen fixation lies between 25°C. to 30°C. according to Russell [1927]. It should be remembered that the soil is quite rich in lime and the supply of organic matter is quite sufficient. Proper aeration was maintained throughout the period of determinations by various field operations mentioned above.

Nitrate and nitrite nitrogen—It is interesting to see the nitrate and nitrite nitrogen changes in the soil under field conditions. Nitrogen in these forms was probably at its lowest on the starting date. But in a month's period it reached its maximum peak in August. The moisture content of the soil during this month was about 24 per cent. and the temperature was rising. The moist soil was stirred on the 28th July after the first determination. Under those conditions nitrification took place very vigorously and gave the maximum figure for nitrate nitrogen. The rainfall during the month of September was more than 5 inches. It not only saturated the surface 9 in. layer of the soil but wetted the lower layers as well. The accumulated soluble nitrates seemed to have been carried down into the lower layer of the soil

by these heavy rains. As most of the plots were bunded, there was no possibility of the loss taking place by surface run-off, but the draining away of the nitrates into the lower layers can account for the sudden fall of nitrate nitrogen in the surface layer in the month of September as the determination was made after most of the rains in that month were received. The moist soil had undergone stirring twice during October on the 1st and 16th of that month and once again in November on the 11th, and as a result the nitrate nitrogen again increased during October and November when a second peak was obtained. It decreased considerably in the following month and remained practically low throughout the cold weather months of January, February and March. Ploughing of the land in March and the subsequent slight wetting of the surface soil raised the nitrate nitrogen in the following three months of April, May and June. Clarke and his associates [1922] also found two peaks for nitrate accumulation under the climatic conditions of the United Provinces.

TABLE X.

The total nitrate and nitrite nitrogen as well as the nitrate and the nitrite nitrogen separately, expressed in milligrams per 100 grams of oven-dry soil.

Date of sampling	Nitrogen as nitrates and nitrites	Nitrate nitrogen	Nitrite nitrogen
July 1930	0·115	0·110	0·005
August 1930	1·324	1·320	0·004
September 1930	0·349	0·340	0·009
October 1930	0·586	0·560	0·026
November 1930	0·980	0·960	0·020
December 1930	0·398	0·350	0·048
January 1931	0·281	0·210	0·071
February 1931	0·268	0·180	0·088
March 1931	0·242	0·180	0·062
April 1931	0·397	0·290	0·107
May 1931	0·410	0·329	0·081
June 1931	0·542	0·390	0·152

It would be seen from the foregoing table that the nitrate nitrogen is the highest in August and the lowest at the start in July, while the nitrite nitrogen is the lowest in August and highest in the month of June 1931. As compared to the quantities of total nitrogen in the soil, the quantities of nitrate and nitrite nitrogens are very small indeed. In every case these forms of nitrogen are less than two milligrams per 100 grams of oven-dry soil. But it may be pointed out that when it is computed on acre area, even restricting to the upper 9 in. layer, every milligram is equivalent to 50 pounds of most available form of

nitrogen for the use of the growing crop. An annual crop of *jowar* removes nearly just this amount. The study of the nitrate curve (Fig. 2) indicates that it has two peaks. The first peak is obtained in August and the standing *kharif* crop if any is likely to be benefited by the readily available nitrogen at that time. The second peak which is gradually reached in November, though somewhat lower, is just in time for the proper nourishment of the *rabi* crop. It must be made clear that the quantities found in the soil are the balance between the nitrification on one hand and the utilization by the crop on the other.

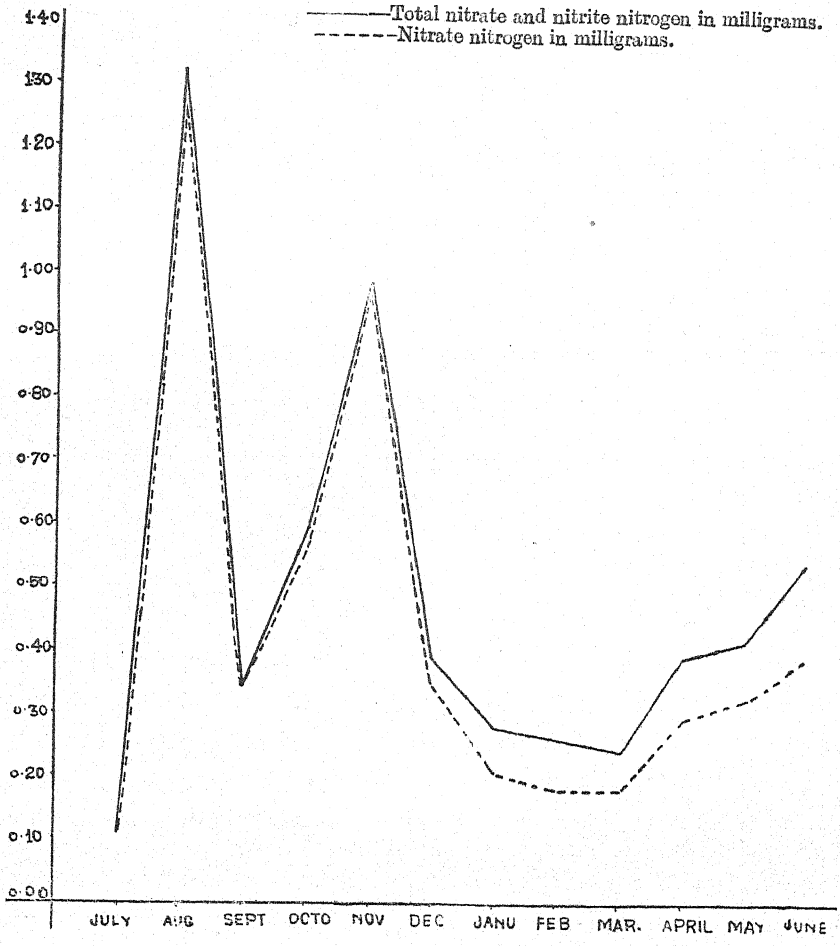


Fig. 2.

III.— Nitrogen changes on a cultivator's plot. Series 2.

The first series of determinations described in Section II was done on a piece of land which was under a crop receiving special care, attention and treatment.

But an ordinary cultivator is not likely to follow the procedure and treatment of land as given there and hence a second series of determinations was made from a piece of land that had a crop grown according to the methods followed by an average Deccan cultivator. The main difference lies in the fact that the cultivator's plot was stirred on less number of occasions and had a higher density of plant population than the specially treated plot and also had some weeds. The area was only one tenth of an acre divided into 4 equal plots and the soil samples were taken at three places in each plot thus obtaining 12 samples from the four plots which were mixed together to obtain a composite sample for determining the nitrogen. The land was ploughed in March and only two harrowings were given in June and July before sowing. After sowing on the 20th of September it was stirred only once in October and there was no more operation till harvest time. The results of the total nitrogen from month to month along with the moisture contents where available are given below.

TABLE XI.

Total nitrogen expressed in milligrams per 100 grams of oven-dry soil and the variation in nitrogen contents when the original is taken as 100 as also percentage of total moisture.

Date of sampling	Total nitrogen	Per centage variation in total nitrogen	Moisture per cent.
July 1930	82·195	100	...
August 1930	82·873	100·77	...
September 1930	88·001	107·00	27·70
October 1930	107·480	130·69	26·13
November 1930	97·398	118·43	21·68
December 1930	133·349	162·15	18·96
January 1931	98·237	119·45	16·91
February 1931	94·283	114·64	13·14
March 1931	78·569	95·53	...
April 1931	97·771	118·52	...
May 1931	86·430	105·09	...
June 1931	63·305	76·97	...

The above statement indicates that even under a cultivator's field conditions there is definite recuperation of soil nitrogen. The maximum rise in nitrogen

contents is nearly the same as on the controlled experimental plots. It is reached in December as in the case of the first series. The chief difference noticed is the sudden fall in nitrogen in one month's period after reaching the maximum, and the amount of nitrogen after this fall is comparatively lower than in the former series. At the end of the year's period the nitrogen left in the soil was about 23 per cent. lower than that with which the series was started.

It may be pointed out that the sudden fall in nitrogen contents and the subsequent lower nitrogen contents may be ascribed to the absence of stirring and consequent insufficient aeration and perhaps also to a drier soil condition. This land on account of insufficient cultivation had not the same fine texture. The experience in the dry-farming experiments is that in cultivator's plot ploughing always gives out large clods which do not easily break up as in the plots specially treated. This cloddy nature resulting in insufficient aeration and weathering may probably account for the lower nitrogen contents.

With regard to nitrogen in the forms of nitrates and nitrites there is no decisive difference in behaviour when compared with that of the first series. The nitrogen in these forms was much higher at the start. The maximum however was reached in August whereas the minimum was reached in December. The nitrites were lowest in August and highest in June as in the former series. The following table gives the quantities of the different forms from month to month.

TABLE XII.

Quantities of the combined nitrate and nitrite nitrogen along with the nitrate and the nitrite nitrogens separately, all expressed in milligrams per 100 grams of oven-dry soil.

	Total nitrite and nitrate nitrogen	Nitrate nitrogen	Nitrite nitrogen
July 1930	0·685	0·68	0·005
August 1930	1·585	1·57	0·003
September 1930	0·481	0·47	0·011
October 1930	0·611	0·53	0·081
November 1930	0·308	0·29	0·018
December 1930	0·239	0·18	0·059
January 1931	0·247	0·18	0·067
February 1931	0·323	0·29	0·033
March 1931	0·269	0·21	0·059
April 1931	0·381	0·29	0·091
May 1931	0·360	0·29	0·070
June 1931	0·661	0·45	0·215

IV.—Effect of previous fallow on nitrogen recuperation.

One more series of the plots in triplicate was under the same treatment of cultivation and crop as that of the first series except for the fact that it was fallow in the previous year, *i.e.*, during 1929. It used to receive one harrowing every month throughout the period of fallow. It was sown in September 1930 and received the same after cultivation as given to the first series. The nitrogen contents of this series, however, is similar to that of the cultivator's plot as can be seen from the following figures.

Table XIII.

Total nitrogen in milligrams per 100 grams of oven-dry soil, percentage change in total nitrogen and the percentage of total moisture in the surface 9 in. layer of soil.

—	Total nitrogen	Percentage change in total nitrogen	Moisture per cent.
July 1930	75·855	100·00	25·12
August 1930	84·762	111·71	26·54
September 1930	84·403	111·24	27·72
October 1930	106·870	140·85	28·42
November 1930	101·961	134·38	23·23
December 1930	128·768	169·71	21·07
January 1931	90·239	118·93	17·69
February 1931	100·946	133·04	16·36
March 1931	91·183	120·17	...
April 1931	109·262	144·00	...
May 1931	92·690	122·16	...
June 1931	67·288	88·68	...

The maximum point of recuperation is reached in December as in the other two series previously described. The nitrogen at the end of the series is lower than that at the start, and is the lowest of the whole series. The great similarity in changes in nitrogen content of the second and the third series consisting of the cultivator's plots and the plots left fallow for one year before cropping can be very well seen from Fig. 3. Both the curves run very much parallel to one another. With

regard to nitrogen in the form of nitrates and nitrites it would be seen from the following figures that, on account of the previous fallow, there is the nitrate accumulation at the start. This is maintained in the following month and then it goes down during wet months of September and October but rises to the highest in November probably due to the stirring of the soil in October and November. Then follows a period of moderate intensity with fluctuations in nitrate contents. With regard to nitrogen in the form of nitrite there is practically no difference in this series and the other two series. The minimum is found in August and the maximum at the end of the series in June.

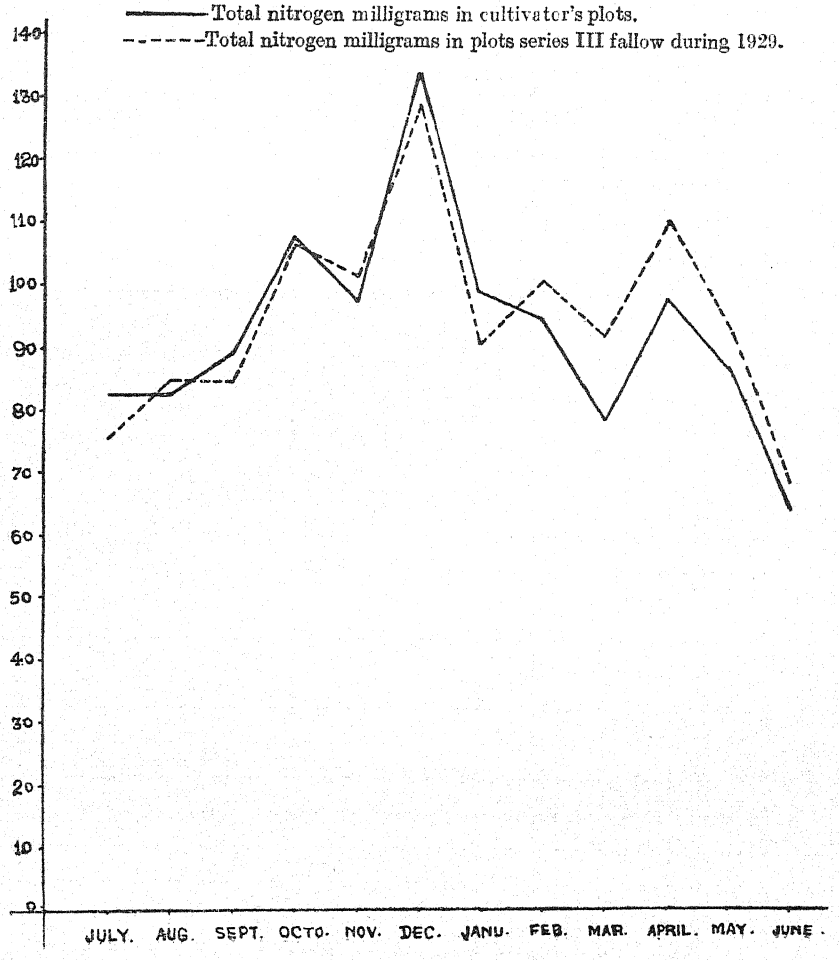


Fig. 3.

TABLE XIV.

Total nitrate and nitrite nitrogen, along with nitrate nitrogen and nitrite nitrogen separately, expressed in milligrams per 100 grams of oven-dry soil.

	Total nitrate and nitrite nitrogen	Nitrogen as nitrate	Nitrogen as nitrite
July 1930	1·065	1·06	0·005
August 1930	1·162	1·16	0·002
September 1930	0·483	0·47	0·013
October 930	0·620	0·54	0·080
November 1930	1·741	1·73	0·011
December 1930	0·358	0·29	0·068
January 1931	0·779	0·73	0·049
February 1931	0·726	0·67	0·056
March 1931	0·353	0·29	0·63
April 1931	0·662	0·58	0·082
May 1931	0·470	0·39	0·080
June 1931	0·578	0·45	0·128

Figure 4 shows the changes of the combined nitrogen as nitrates and nitrites from month to month in the cultivator's plot and in the plot left fallow in the year previous to that of the experiments. It may be pointed out that the curve showing the readily available nitrogen in the cultivator's plots is very much lower in comparison with that of the fallow throughout the active growing period of the *jowar* crop. The high peak in August is of no use on account of the absence of a crop at that time. The maximum peak of the fallow series is reached just at a time when the vigorously growing crop needs it. Again in the fallow series the available nitrogen remains high throughout the growing period of the crop which thus accounts for the higher yields of the fallow series in contrast to that of the cultivator's plot series.

The results of the investigations with regard to nitrogen contents of all the series so far described can be considered together as of one group, as none of the plots received any kind of manure during the period of seven years. They may be summarised as follows:—

(1) The nitrogen content of the soil is not a constant quantity. It fluctuates from month to month.

(2) There is a definite recuperation of nitrogen by soils and this is far more pronounced in fields than indicated under laboratory conditions. Recuperation continues for a much longer time under field conditions than was observed under laboratory conditions.

(3) The maximum peak of the curve is reached sometime in December under the conditions of the experiments while the minimum is found either in June or July. In December the combination of the moisture contents of the soil and the soil temperature is the most favourable in the whole year for nitrogen recuperation.

(4) The highest increase in the nitrogen contents amounts to 62 to 69 per cent. of the original nitrogen.

(5) Moisture, soil temperature and soil aeration due to the stirring of the soil by harrowing or interculturing have great influence in changing the nitrogen contents of the soil.

———Total nitrate and nitrite nitrogen milligrams in cultivator's plots.
------Total nitrate and nitrite nitrogen milligrams in plots series III fallow during 1929.

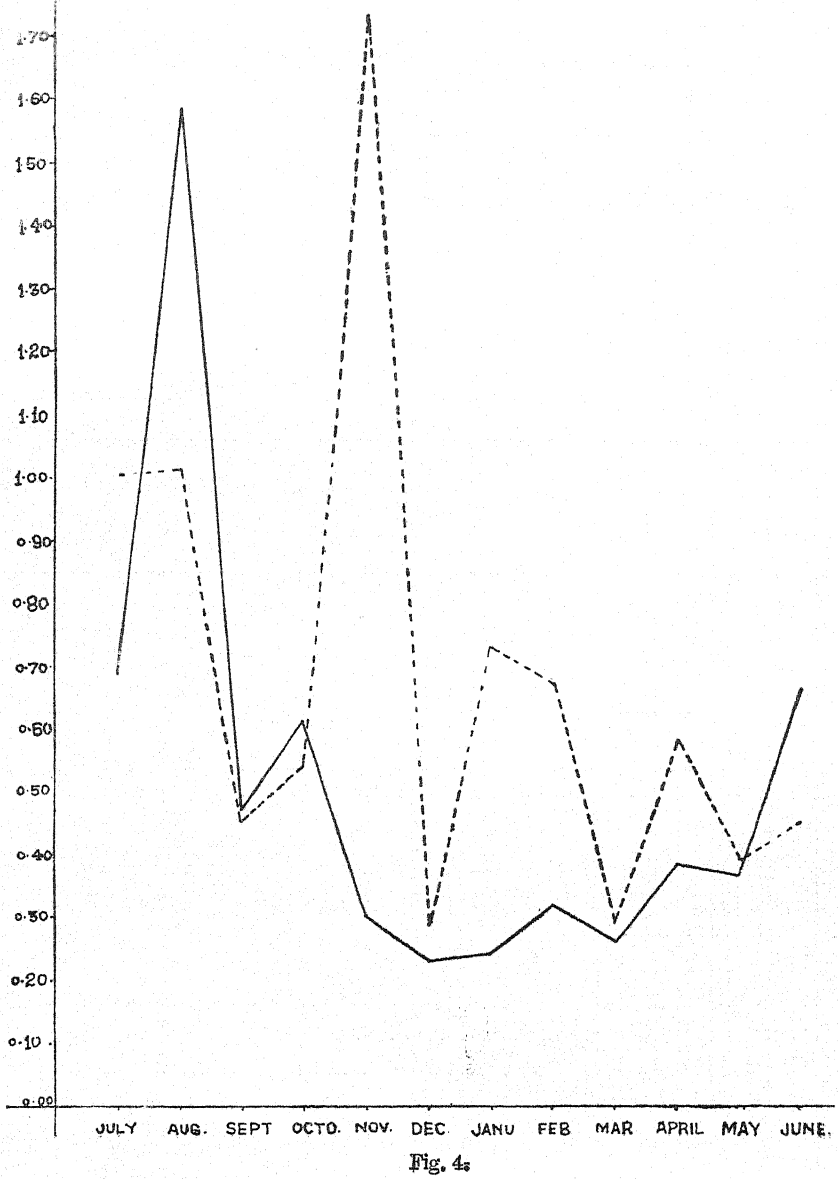


Fig. 4.

(6) The nitrate nitrogen seems to reach its maximum in August except in the case of the fallow series where it is reached somewhat later.

V. Effect of the addition of organic matter to the soil on its nitrogen recuperation, series 4, 5 and 6.

Three more sets of plots (Series 4, 5 and 6) were under observation for determining the changes in total nitrogen contents as well as the nitrogen in the form of nitrates and nitrites.

The first of these sets consisted of 3 plots which had received farmyard manure at varying rates in alternate years.

The second set had additions of green manure in the form of *sann* (*Crotalaria juncea*) during the year of experimentation.

The remaining set had the addition of *sann* every alternate year from 1924 to 1928. This series is intended to find the residual effect of organic matter previously added.

Table XV.

The year, the quantity of material added and the equivalent amount of nitrogen added per acre.

Series 4, F. Y. M. added in alternate years			Series 5, *sann* added only once			Series 6, *sann* added in alternate years		
Year of addition	Quantity of F. Y. M. added per acre lbs.	Addition of equivalent N per acre lbs.	Year	Quantity of green *sann* added lbs.	Addition of equivalent N per acre lbs.	Year of addition	Quantity added per acre lbs.	Addition of equivalent N per acre lbs.
1926	8,600	46·4	..	..	..	1924	16,000	67·2
1928	8,000	64·8	..	..	..	1926	7,600	32·0
1930	8,000	48·0	1930	2,080	11·9	1928	5,400	22·7
		159·2						121·9

In contrast to the first three series of experiments attempt was made to replenish the soil nitrogen removed annually by the crop, by the addition of well-rotted farmyard manure or by addition of green plant matter to the soil as green manure in the form of *sann*.

The green manure crop was grown *in situ* from June to August and then buried by the middle of August, in 1926, 1928 and 1930; while in 1924 the crop was grown outside, pulled and added to the plots. The following table gives the quantity of total dry matter of *jowar* crop produced on these manured plots and the quantity of nitrogen removed annually by these crops. For comparison results of dry matter produced from the unmanured plots described in the first series are also given.

TABLE XVI.

Pounds of dry matter of rabi jowar *produced from year to year and the pounds of nitrogen removed per acre in three series of plots receiving different treatments.*

Years	Unmanured plots. Series 1		Plots to which farmyard manure was added in alternate years. Series 4		Plots to which green manure was added in alternate years. Series 6	
	Total dry matter per acre lbs.	Nitrogen removed per acre lbs.	Total dry matter per acre lbs.	Nitrogen removed per acre lbs.	Total dry matter per acre lbs.	Nitrogen removed per acre lbs.
1924 . .	4,217	42	3,673	36	4,741	47
1925 . .	2,148	21	1,766	17	2,577	25
1926 . .	2,467	24	2,744	27	2,313	23
1927 . .	4,619	46	3,026	30	3,964	39
1928 . .	3,423	34	3,036	30	4,806	48
1929 . .	2,541	25	4,005	40	3,442	34
1930 . .	2,534	25	3,853	38	3,143	31
Total .	21,949	217	22,103	218	24,986	247

It would be seen from the above table that the addition of farmyard manure in alternate years does not make much difference in the total yield of dry matter. In the case of green manure the total quantity of nitrogen removed by seven annual crops is 247 pounds. But it should be noted that in the case of farmyard manure 159 lbs. of nitrogen was added while in the case of green manure 122 lbs. of nitrogen was added. Series 5 consisted of duplicate plots only and received green manure only once during the year of experiment. On account of insufficient early rains, the crop grown was very poor and the addition of organic matter and therefore nitrogen was very small. Even then the changes in the total nitrogen contents of this plot are worth comparison with those of the plots of series 4 and 6 which had received additions of farmyard manure, or *sann* in previous years.

In the series receiving farmyard manure the total gain is as much as 85 per cent. and in the series to which *sann* was added the total increase is 93 per cent. over the original. In these series the nitrogen left in the soil at the end of the

year's period is much higher than at the start. The greater activity of both these series as compared to that of unmanured plots is further noticed in the fact that the rise in nitrogen contents is seen continuously from the very first month until the maximum peak in the curve is reached. The presence of organic matter naturally changes some of the important physical properties. These changes are bound to affect the nitrogen changes in the soil. Further, ups and downs or fluctuations in the total nitrogen contents are noticed but the nitrogen at the end is much above that at the start. These changes are seen from Fig. 5.

TABLE XVII.

Quantities of total nitrogen in milligrams per 100 grams of oven-dry soil, along with percentage change in total nitrogen from month to month in the series 4, 5 and 6 which received additions of organic matter.

Month	*Series 4.* Addition of F. Y. M. in alternate years		*Series 5.* Addition of *sann* as green manure in 1930		*Series 6.* Addition of *sann* in alternate years of 1924, 1926 and 1928	
	Milligrams of total N per 100 grams of soil	Changes in N contents on the basis of 100 as the N at start	Milligrams of total N per 100 grams of soil	Changes in contents on the basis of 100 as the N at start	Milligrams of total N per 100 grams of soil	Changes in contents on the basis of 100 as N at start
July . .	62·04	100·00	55·86	100·00	71·55	100·00
August . .	95·44	153·75	68·29	122·24	98·43	137·14
September .	98·15	158·12	76·99	137·78	77·11	107·76
October . .	95·48	153·82	108·03	193·33	104·17	145·57
November .	114·87	185·05	88·29	158·01	109·45	153·00
December .	95·23	153·41	105·75	189·27	130·71	182·68
January . .	89·73	144·55	72·27	129·36	111·89	156·37
February .	98·36	158·45	78·70	148·26	102·50	143·24
March . .	104·74	168·73	87·15	156·21	89·83	125·54
April . .	109·98	176·59	99·69	178·45	111·06	155·28
May . .	88·16	141·28	96·52	172·74	100·65	140·67
June . .	104 77	168·04	85·02	152·66	99·23	138·68

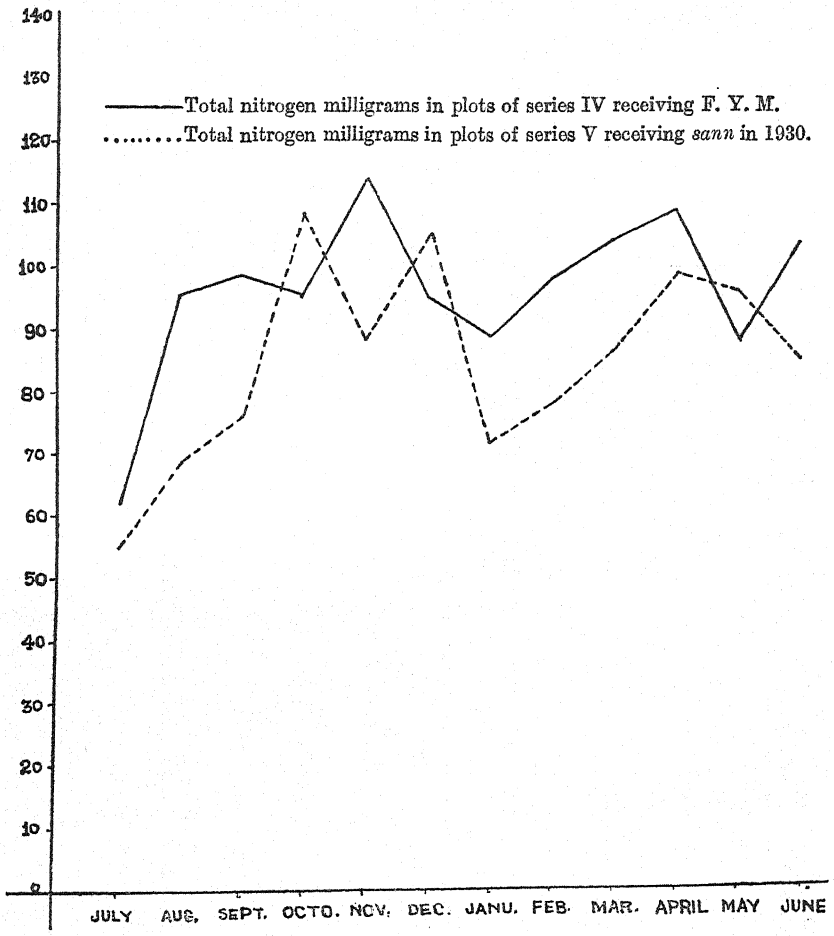


Fig. 5.

Series 6 which had received additions of organic matter in the form of *sann* in three alternate years shows a great activity, the largest increase in nitrogen content is more than 82 per cent. over the original. But excepting this large increase the fluctuations in the curve obtained are very similar to those obtained in the unmanured series I, which was subjected to only frequent cultural operations without any additions of manure. An increase in nitrogen contents is obtained in the first month but there is a sudden fall in the next. The maximum peak in the curve is again reached in December as in the unmanured series and then the nitrogen drops gradually giving a fluctuating curve in the later period. It may be

pointed out that additions of *sann* were made in 1924, 1926 and 1928. There was no addition in 1929 and 1930. During this period of two years the active effect of organic matter seems to have very much decreased and the only residual effect left was mainly seen in the great increase in the nitrogen content. The curve in other respects is very much similar to that of the unmanured series. Figure 6 illustrates this comparison very clearly.

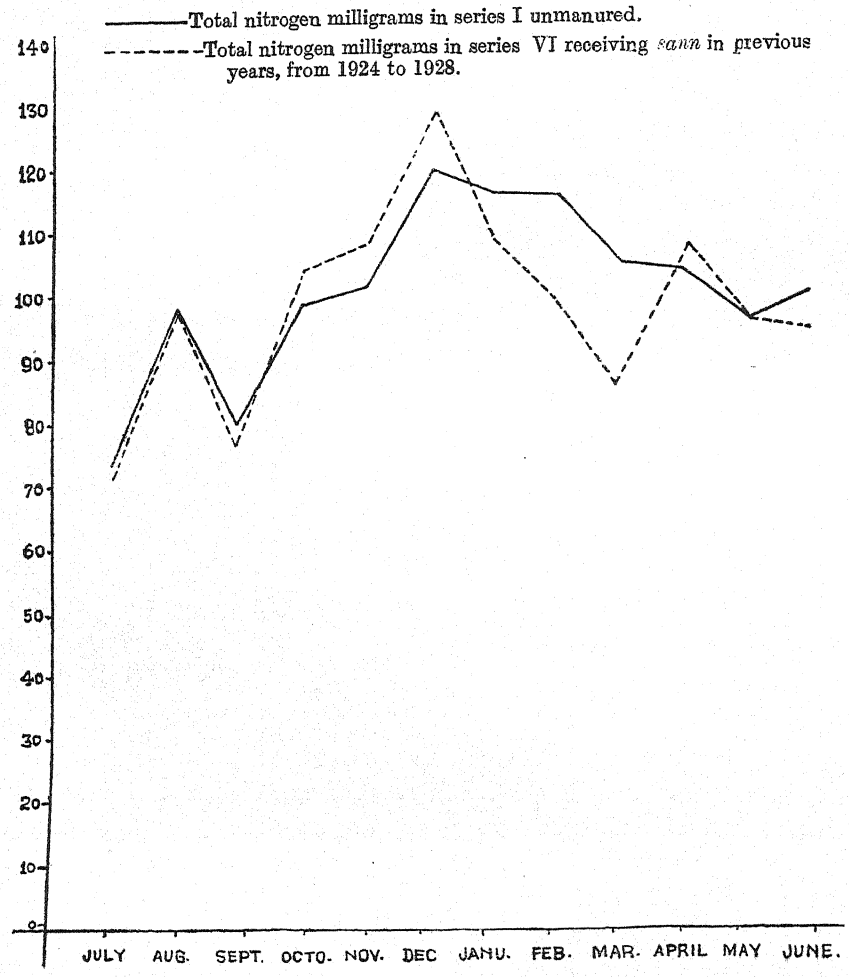


Fig. 6.

Nitrate and nitrite nitrogen of manured series.—From the tabular statements giving the nitrate and nitrite nitrogen from month to month in all the three series which received organic matter sometime or the other during the period of seven years, it would be seen that the series receiving farmyard manure and fresh *sann* in the year of experiment show more or less the same fluctuations. In the case of plots receiving fresh *sann* there was at the start a large quantity of nitrates which went down in August due to the putting in of the green manure. This however increased in the following month and reached the maximum peak in October. It began to fall in November and then remained at a low level throughout the following seven months. But in the case of farmyard manure the quantity of nitrates was lowest at the start in July. The nitrates began to rise gradually from the very next month and rose to the maximum in November and then dropped down by sixty per cent. and then remained practically low throughout the following month. The nitrites in both the series were the lowest at the start and reached the maximum at the end of the series, *viz.*, in June.

Series 6 which had received *sann* green manure three times since 1924 and had only the residual effect in the year of experiment showed the nitrate curve very similar to that of the series receiving no manure. Starting with low nitrate contents in July, the maximum was reached in the month of August which then dropped in the next month and gave a second peak of rise in November and then after dropping down in December remained at a low level throughout the following six months. The nitrites were lowest at the start in July and were highest in May and remained practically at that level in the following month.

As the sum of these forms of nitrogen indicates the activity in the soil for converting highly synthesised form of nitrogen into the simpler and available forms it seems that the most active period in all cases is restricted to a period from July or August to November according to the most suitable conditions for available moisture, temperature and aeration. Figure 7 gives the comparative changes in the total nitrate and nitrite nitrogen in the three series receiving organic matter for the purpose of manuring.

————Total nitrate and nitrite nitrogen milligrams, F. Y. M. series IV.
—·—·—·—Total nitrate and nitrite nitrogen milligrams, *sann* in 1930, series V.
– – – – – –Total nitrate and nitrite nitrogen milligrams, *sann* in previous years, from 1924 to 1928, series VI.

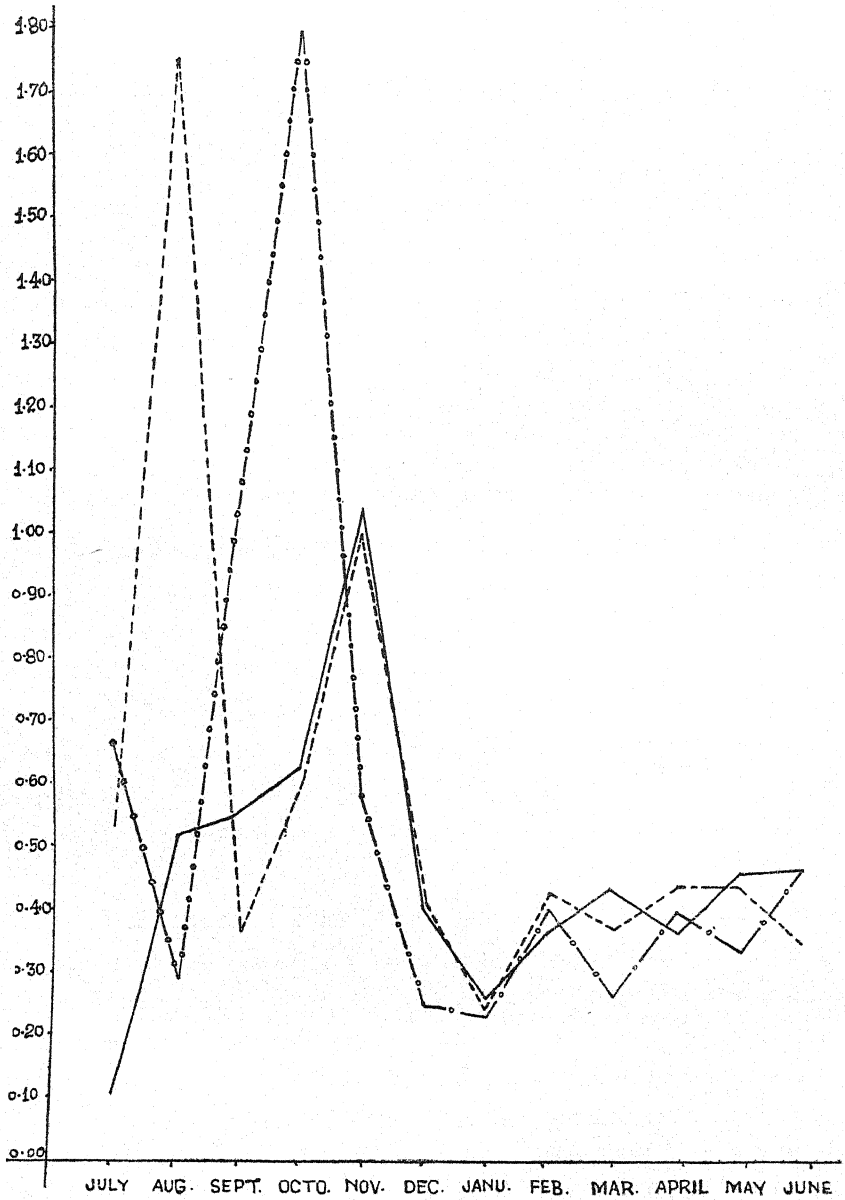


Fig. 7.

TABLE XVIII.

Total nitrate and nitrite nitrogen combined, and nitrate and nitrite nitrogens separately, in milligrams of nitrogen per 100 grams of soil.

Month	Series 4, addition of F. Y. M.			Series 5, *sann* in 1930			Series 6, *sann* in alternate years from 1924 to 1928		
	Total nitrate and nitrite nitrogen	Nitrate	Nitrite	Total nitrate and nitrite nitrogen	Nitrate	Nitrite	Total nitrate and nitrite nitrogen	Nitrate	Nitrite
July . .	0·092	0·087	0·005	0·665	0·66	0·005	0·536	0·53	0·006
August .	0·515	0·510	0·005	0·295	0·29	0·005	1·747	1·74	0·007
September .	0·550	0·540	0·010	1·076	1·06	0·016	0·360	0·35	0·010
October .	0·622	0·590	0·032	1·780	1·69	0·090	0·590	0·52	0·070
November .	1·077	1·07	0·007	0·589	0·58	0·009	0·992	0·97	0·022
December .	0·403	0·350	0·053	0·255	0·21	0·045	0·429	0·38	0·049
January .	0·269	0·21	0·059	0·234	0·18	0·054	0·246	0·18	0·066
February .	0·368	0·29	0·078	0·406	0·35	0·056	0·422	0·34	0·082
March . .	0·434	0·37	0·064	0·267	0·21	0·057	0·374	0·29	0·084
April . .	0·365	0·29	0·075	0·391	0·29	0·101	0·431	0·35	0·081
May . .	0·457	0·34	0·117	0·330	0·27	0·060	0·437	0·33	0·107
June . .	0·465	0·35	0·115	0·461	0·35	0·111	0·345	0·24	0·105

The results of the investigation of the second group of plots dealing with three series receiving organic matter added in the form of farmyard manure and green manure of *Crotalaria juncea* may be summarised as follows :—

(1) Nitrogen contents of the soils treated with organic matter show changes very similar to those observed in untreated soils. (2) The total increase or gain in nitrogen content over the original is, however, much greater in these series than in the series receiving no organic matter. (3) The time of maximum increase is a month or two earlier in the case of addition of farmyard manure or of *sann* in the year of experiment. (4) Where the addition of *sann* was done two years before the experiment the activity and changes in nitrogen contents are very similar to those of unmanured series. (5) There is no substantial difference in the nitrogen changes in the form of nitrates and nitrites except the fact that the maximum peak of nitrate contents is reached later in October or November in cases where additions of orga-

nic matter are done freshly. In the case of residual organic matter, the nitrate and nitrite nitrogens behave exactly as those in the unmanured series.

Conclusions.

(1) Definite recuperation of nitrogen takes place in the soils under field conditions in the dry farm tracts of the Bombay Deccan. Wetting of the soil by the monsoon rains and the subsequent partial drying and heating during the dry spells of the monsoon seems to be favourable for starting the recuperation process. Better cultivation of the land helps to maintain the nitrogen contents of the soil fairly high and does not allow a sudden drop after the maximum peak is reached. The recuperation is much more pronounced and continues much longer in the fields than what was observed under laboratory conditions.

(2) Addition of organic matter in the form of farmyard manure or *sann* (*Crotalaria juncea*) increases the recuperation power of the soil and maintains the nitrogen contents at a fairly high level.

(3) The maximum rise of the total nitrogen contents takes place at a time when the soil moisture is about 20 per cent. and the soil temperature nearly at 30°C. Stirring of the soil by cultivation helps the recuperation process.

(4) The quantities of nitrates and nitrites also fluctuate between a certain range. Cultivation and addition of organic matter give two peaks of nitrate nitrogen which are directly useful for the growing crop. Fallowing also results in increasing the amount of nitrates and giving two periods of increase during the crop period. The nitrate nitrogen, though it forms a very small proportion of the total, being in the most readily available form, can prove to be effective in giving increased yields.

(5) The investigations of the nitrogen contents of the soil under field conditions for a period of one year from month to month have shown very conclusively that the nitrogen content of the soil is not a stable or a constant quantity. There is a range in every soil depending upon such factors as the moisture, temperature and aeration, which in their turn are dependent upon the climatic factors. Hence the determination of nitrogen contents of a soil without any attention to the time or season of sampling or other concomitants like the moisture and temperature, is not likely to throw much light on the fertility of the soil as far as this ingredient is concerned.

(6) Different groups of soils may have different ranges of nitrogen contents depending upon the presence or absence of other soil constituents like organic matter or lime. Such ranges will have to be determined in different soils to enable the chemist to judge as to the adequacy or otherwise of the quantity found taking

into account the season of sampling. Further work in nitrogen recuperation must therefore be in this direction.

REFERENCES.

Clarke, G., Banerjee, S. C. and Husain, M. N. and Qayum, A. (1922). *Agric. J. Ind.* **17**, 463.

Greaves, J. T. (1918). *Soil Sci.* **6**, No. 3.

Russell, E. J. (1927). Soil Conditions and Plant Growth. Fifth edition, page 274.

Sahasrabuddhe, D. L., and Daji, T. A. (1925). *Mem. Dept. Agric. Ind., Chem. Ser.* 8, No. 5.

Sahasrabuddhe, D. L., and Ghatikar, B. H. (1931). *Ind. J. Agric. Sci.* 1, 631.

ON THE VARIATION OF CERTAIN CHARACTERS OF COTTON IN RELATION TO THE POSITION OF SEEDS IN A LOCK.

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WITH A

STATISTICAL NOTE

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(With two text-figs.)

INTRODUCTORY.

The present work was undertaken in order to determine the variations in the seed and the lint weights due to the position of the seed in the lock. The materials selected for this purpose were the well-opened bolls of the Punjab-American cottons, *viz.*, (*a*) The Early Strain, (*b*) 4 F, and (*c*) 289 F, picked *at random* in November 1929, from different plants from the Physiology Plot of the Cotton Research Farm, Lyallpur. A detailed investigation, involving the fibre length and the fibre weight determinations, was carried out on the Early Strain alone. For the sake of com-

parison the badly opened bolls* of all the three cottons were also investigated with regard to the main problem.

Literature bearing on the subject of this paper is rather scanty. Turner [1929, 1] remarked that " difference in lint weight or seed weight may indeed be due to differences in the situation of seeds in a single boll ". Ramanatha Iyer [1929] has shown: (i) that the seed weights and the lint weights are the highest near the basal position, (ii) that the apical seeds give the smallest seed and lint weights and (iii) that the ginning percentages are higher in the lower portion of the lock. Balls [1915] also has done much work on the relationship between the lint weight and the seed weight in raw cotton. He concluded that there is a fair amount of relationship between the lint weight and the seed weight, in as much as the same cause which affects one also affects the other. Kearney [1928] has extended Ball's work and has shown that the lint weight and the seed weight are correlated even in the individual bolls of Pima Cotton.

Sampling.**

In order to obtain representative samples the following procedure was adopted. One thousand locks were collected at random (irrespective of the number of locks in the boll and the position of the boll on the plant) from among the open bolls from various plants in each variety. From out of these a random selection of 200 locks was made; these 200 locks were then separated at random again into 10 lots of 20 locks each.

In order to be able to tabulate systematically the results for each position, the different seeds in a lock were numbered serially, as shown in Fig. 1. While thus numbering the different seeds, each lock was placed on a table with the basal end near the observer and the funicles of the seeds uppermost.

* The badly opened bolls are easily distinguished from the well-opened bolls by their appearance. As the causes leading to the bad opening of bolls Trought (*Ind. J. of Agric. Sci.* Vol. I, Part III, page 335) suggests that " unfavourable conditions, climatic and biological, overlap sufficiently to produce a general pathological state in the plant, which shows finally in the failure of bolls to develop properly ".

** This method of sampling was suggested to the author by Mr. Trevor Trought, Cotton Research Botanist, Lyallpur.

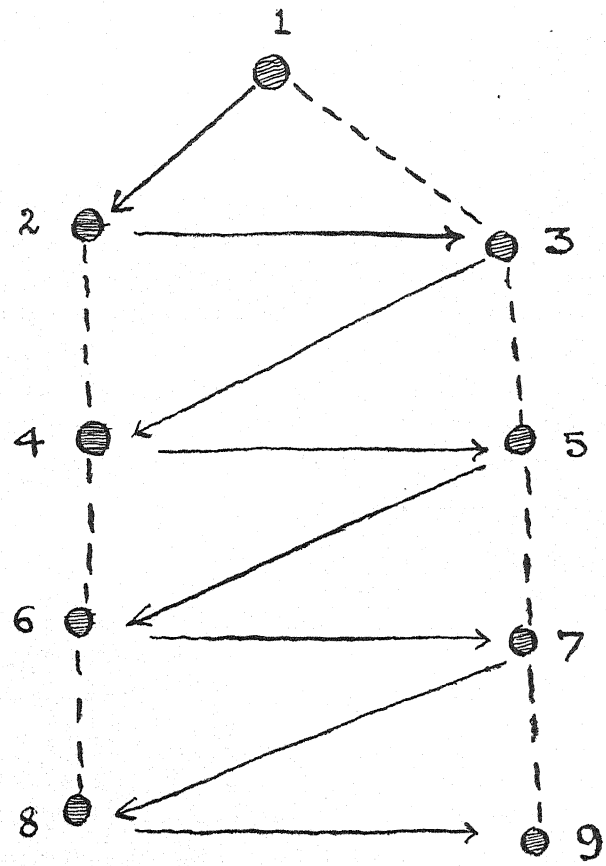


Fig. 1.

Experimental procedure and sources of error.

The measurements were carried out about a week after the collection of the samples in order to allow for the drying of the lint. The actual procedure adopted was as follows :—

At first the fibres on the seeds in each lock were carefully teased apart with a microscopist's dissecting needle and equally carefully cleansed of all adhering dirt and leaf particles with a good pair of forceps. The lock was then spread out on the table with the funicles facing the observer, as in Fig. 1, and the seeds, with lint intact, were slowly pulled apart until dissociated from one another. The seeds thus detached were left on the table in the same positions relatively as within the lock

itself, and then another lock was similarly treated. This process was continued till the seeds of all the 20 locks in a lot had been so detached. The seeds belonging to the same position were then collected together from all these twenty locks. Afterwards all the 20 seeds with lint attached were weighed separately for each position in a balance of the steelyard type reading up to the tenth of a gram. The lint was then separated by pulling the hairs off from the seeds by hand, taking care to safeguard against damages as far as possible. The seeds were then weighed again in the same balance. The difference between this weight and the former weight gave the total weight of the lint. Hence the weight of one seed as well as the lint weight per seed was obtained. The lint separated was also weighed independently to serve as a check on the lint weight otherwise determined. The same procedure was adopted in the case of each lot.

The lint of Early Strain was then utilised in determining (1) the fibre weight (by the " cutting " method), and (2) the average fibre length (by Balls' sledge sorter).

The chief sources of error in the conduct of these experiments were as follows :—

(i) the loss or the gain of fibres by a seed in the process of its extraction from the lock ;

(ii) retention of some small parts of the fibres by a seed during separation of the lint by hand ;

(iii) absorption of grease and moisture by the lint during handling ; and

(iv) the effect of humidity during the period of the experiments.

The first two sources of error cannot of course be completely eliminated, but they should in the present case be very small owing to the great amount of care taken at every step.

The third source of error was reduced to a minimum by working with hands washed with soap and water and dried in the air.

With regard to the last source of error it may be stated that the relative humidity inside the Cotton Research Laboratory, Lyallpur, between 10 A.M. and 2 P.M. in November 1929 (when the actual measurements of the seed weight and the lint weight were carried out) was as low as about 27 per cent. on the average. The range of variation within the period of work was 15 per cent. to 40 per cent., while the extreme range of variation on a single day was 15 per cent. to 29 per cent. Moreover the time of exposure to the atmosphere for any one sample under experiment was also small, being only a few minutes. It is highly probable, therefore, that no serious harm was done; for, according to Balls [1928] the maximum percentage by weight of water absorbed at 40 per cent. relative humidity after 2 days' exposure is only 5·34 per cent.

Discussion of the results.

The results will be conveniently discussed in two stages. In the first stage the main problem, *viz.*, the seed and the lint weights for all the cottons, will be considered; in the second stage all the results for the Early Strain alone will be discussed.

(a) *The main problem.*

In comparing the values for the well-opened and the badly opened bolls (Table I), it is found that for the apical position the seed weight as well as the lint weight have got low values for both kinds of bolls. From the graphical representations (Fig. 2) of these results a tendency towards a maximum value for the seed weight curves near about the basal positions can be fairly distinguished in the case of the badly opened bolls. The lint-weight curves for the well-opened bolls also exhibit such a tendency to a slight extent. Ramanatha Iyer [1929] working with *G. hirsutum* and *G. cernuum* also observed a similar phenomenon.

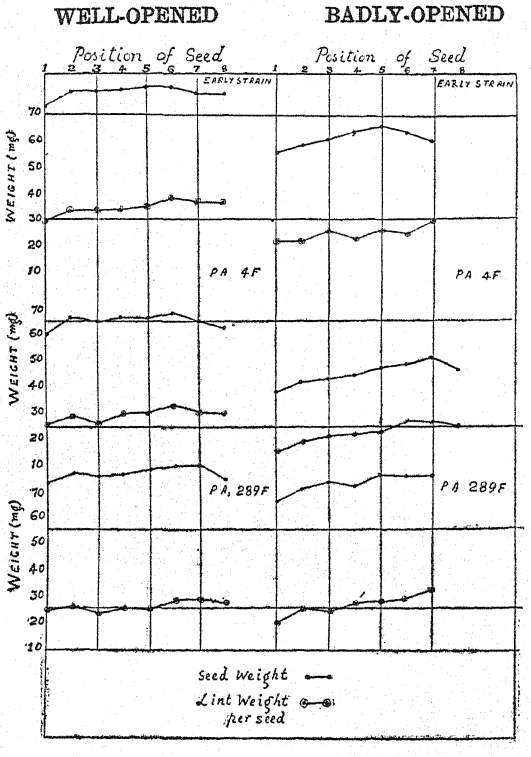


Fig. 2.

TABLE I.

Position of seed in the lock	A.—Well-opened bolls												B.—Badly opened bolls					
	Early Strain				P.-A. 4 F				P.-A. 289 F				Early Strain		P.-A. 4 F		P.-A. 289 F	
	Seed weight (mg.)	Standard error	Lint weight per seed (mg.)	Standard error	Seed weight (mg.)	Standard error	Lint weight per seed (mg.)	Standard error	Seed weight (mg.)	Standard error	Lint weight per seed (mg.)	Standard error	Seed weight (mg.)	Lint weight per seed (mg.)	Seed weight (mg.)	Lint weight per seed (mg.)	Seed weight (mg.)	Lint weight per seed (mg.)
1	73	1·2	29	1·5	60	1·5	26	1·7	73	1·7	25	0·8	54	20	37	14	65	19
2	79	1·9	33	1·4	66	1·8	29	1·4	77	2·0	26	0·9	57	20	41	18	70	24
3	79	1·8	33	1·5	65	1·1	26	1·5	76	1·8	24	0·9	59	24	42	20	73	23
4	79	0·9	33	1·1	66	1·2	29	0·9	76	1·5	25	1·3	62	21	43	21	71	26
5	80	2·0	34	1·6	66	1·0	30	1·3	78	1·4	25	0·9	64	24	46	21	75	27
6	80	1·7	37	1·6	68	0·9	32	1·7	79	1·2	28	1·2	62	23	48	25	75	28
7	78	1·7	36	1·0	65	1·1	30	1·8	80	0·9	28	1·6	59	27	50	25	75	31
8	77†	..	35†	..	62‡	1·7	29‡	3·0	74‡	..	27‡	..	..	..	45	24	..	..
9	..	..	..	..	..	..	..	..	78*	..	25*	..	..	..	..	..	..	..

The phenomenon that the apical seeds have always the least weight is quite intelligible from the point of view of nutrition. But it is interesting to note that generally the basal seeds also have lower weights than the intermediate ones. These variations in the weights of single seeds in individual locks are very interesting and appear to fall in line with the work on other field crops. As an analogous case it may be mentioned that Engledow and Wadham [1923] in their investigations on the yield in cereals determined the mean weight of barley grains on each side of the ear in a random sample of 100 ears. They numbered the grains along the rachis from tip to base as R 1, L 1, R 2, L2..... and so on. They found that proceeding from the tip to the base the weights of the grains increased rapidly to a maximum for a position nearer the base than the tip, and then decreased markedly towards the basal positions.

Thus the analogy with the variation in weights of single seeds in different positions in a lock of cotton seems to be complete. It is very likely, as Afzal and Trought [1932] suggest, that the cause of this interesting phenomenon may be traced to the supply of nutrition to the various parts of the boll due to any of the following causes :—

"(1) The distribution and size of the vascular bundles in the boll.

† Available for 2 lots only. Total number of seeds = 24 in place of 200 (expected).

‡ Total number of seeds for the 8th position = 132 instead of 200 for P.-A. 4 F; and 70 instead of 200 for P.-A. 289 F.

* Only 16 healthy seeds in place of 200 (expected) were available for the 9th position. These values are rejected

(2) the rate of flow of nutrients to the different portions of the boll.

(3) the internal pressure set up by a possibly differential rate of growth of locks and the epicarp of the boll.

(4) the competition for nutrients between different seeds inside the boll."

It may be noted that these authors have found that the number of motes is high towards the extreme portions of a lock, there being a minimum somewhere near the middle. These conclusions are in line with the occurrence of lower seed weights at the extremities of locks than in the intermediate positions.

Now, since the 200 seeds for each position were tested in 10 lots, we have 70 readings on the whole on account of the 7 positions taken together, for either the seed weight or the lint weight for each variety. Taking all these 70 pairs of readings without regard to positions, we get the following coefficients of correlation between seed weight and lint weight

(1) Early Strain	$r=+0{\cdot}45$	$\pm$ 0·065
(2) 4F	$r=+0{\cdot}33$	$\pm$ 0·071
(3) 289F	$r=+0{\cdot}528$	$\pm$ 0·059

Since these coefficients of correlations are based on 70 pairs of readings, it can be assumed that the ordinary method of ' probable error ' calculation and the test of significance from normal distribution tables can be applied. It is found that the ratio of the correlation coefficient to its probable error is more than 3 in all the cases. So we can say that on the whole the seed and the lint weights are correlated.

Correlation between seed weight and lint weight has been found to exist both by Balls [1915] and Kearney [1928], the former working with raw cotton and the latter with single bolls. Afzal [1930] working with a hybrid at Trinidad, found that the seed index was " very highly correlated " with lint index, the coefficient of correlation being $r=+0{\cdot}710\pm0{\cdot}024$.

It is clear from Table I that the seed weights and the lint weights of all the three varieties are better for the well-opened bolls than for the badly opened ones. As the bad opening of the bolls is due to a general physiological debility of plants [Trought 1931], a reduction in the weights of lint and seed is to be expected.

(b) *The Early Strain.*

The Early Strain has been investigated with regard to the mean fibre weight per unit length* and the average fibre length† in addition to the seed weight and the lint weight for each position of the seeds in a lock. The weight of a fibre of

* The fibre weight for each position was obtained by weighing 1 cm. length of fibres in 5 lots of 250 fibres each, thus making up a total of 1250 fibres for each position. Each lot of 250 fibres was collected from five independent tufts of hair from a sliver after cutting. The mean sensitivity of the quartz microbalance was 0·050 mg. per div.

† 2 Balls sorter tests from two independent slivers were made.

average length, the number of hairs per seed and the ginning percentage for each position have also been calculated from the data available. The results appear in Table II.

Analysing these results, it is found that the fibre weight per unit length for the apical seed is the lowest, whereas the fibres from the seeds nearest the base have comparatively higher weights per unit length than the fibres from any other seed in the lock. The fibre weights per unit length for the intermediate positions are intermediate in value and, within the limits of probable error,* fairly constant.

The average fibre length, however, is practically the same for all the positions. Thus the present experiments are not in accord with V. Ramanatha Iyer's [1930] expectation regarding " differences in lint lengths of seeds according to their position in the lock ".

Figures given in Table II indicate that when the seed weight is considerably low, the weight of a fibre of average length also becomes low.

TABLE II.

Early Strain (well-opened).

	Experimental				Calculated		
I Position of seed in the lock	II Seed weight (mg.)	III Lint weight per seed (mg.)	IV Average fibre length by Balls' sorter (cms.)	V Mean fibre weight per cm. ($\times 10^{-6}$ gm.)	VI Weight of a fibre of average length ($\times 10^{-6}$ gm.) (Col. IV × Col. V)	VII Mean number of hairs per seed	VIII Ginning percentage
1	73	29	2·32	1·78	4·13	7022	28·3
2	79	33	2·36	1·85	4·37	7551	28·9
3	79	33	2·41	1·94	4·68	7041	29·0
4	79	33	2·36	1·95	4·60	7174	29·6
5	80	34	2·35	1·96	4·61	7375	29·6
6	80	37	2·35	2·07	4·86	7613	31·6
7	78	36	2·31	2·10	4·85	7423	31·1
8	77	35	..	..	..	..	..

* " The probable error of a single observation of fibre weight lies between 3 and 6 per cent. for most Indian cottons."—A. J. Turner, "Technological Report on Standard Cottons 1929 ", p. 18.

From his data on single seeds, Turner [1929, 2] concluded that ginning percentage bears no direct relationship to the number of hairs per seed. He also found that the number of hairs per seed does not bear any significant correlation to the seed weight.

The present experiments appear to confirm these conclusions. But no indication could be obtained from the present work with the Early Strain regarding the "association of high ginning percentage with low seed weight" as shown by Turner [1929,5] to exist in the case of A. 19 and 4-F.

It may be mentioned here that Turner did not select his seeds as in the present case with reference to their positions in a lock, nor did he distinguish between the well-opened and the badly opened bolls. What he did was merely to reject the diseased seeds [Turner, 1929, 4].

The ginning percentages, as calculated from the present data, show an increase towards the basal positions. The same phenomenon is discernible in the case of the other two cottons also (Table IV). This may probably be due to the following causes, alone or in combination :—

(1) the increased weight of individual hairs through extra thickening*.

(2) the increased number of hairs per seed (Table II).

(3) a diminution in the seed weight (Table II).

TABLE III.

$$\text{Coefficients of variability} = \frac{100 \times \text{standard deviation}}{\text{mean}}$$

(*Early Strain, well-opened*).

Position of seeds	Coefficient of variability for seed weight	Coefficient of variability for lint weight	Coefficient of variability for ginning percentage: $\frac{100 \times \text{lint weight}}{\text{seed weight} + \text{lint weight}}$
	Per cent.	Per cent.	Per cent.
1	5·4	17·0	13·2
2	7·5	13·5	10·9
3	7·3	14·1	9·4
4	3·8	10·8	9·6
5	8·2	15·4	11·6
6	6·8	13·5	12·4
7	7·0	8·8	9·9

* Balls, as quoted by Turner in the Tech. Bull. (I. C. C. C.) Ser. B, No. 4, p. 8, does not appear to be agreeable to this view with regard to his work on raw cotton.

TABLE IV.

Ginning percentages (well-opened bolls).

Position of seed in the lock	Early Strain	4-F	289-F
	Per cent.	Per cent.	Per cent.
1	28·3	30·2	25·5
2	28·9	30·5	25·2
3	29·0	28·6	24·0
4	29·6	30·5	24·8
5	29·6	31·3	24·3
6	31·6	32·0	26·1
7	31·1	31·6	25·9
8	—	31·9	—

In Table III are given the coefficients of variability for the seed weights, the lint weights and the ginning percentages corresponding to each position for the Early Strain. It is evident therefrom that the seed weights of the Early Strain are the least variable whereas the lint weights are the most variable. But the ginning percentage, which is a complex character involving both the seed and the lint weights, shows less variation than the lint weight for different positions. In explanation of such a phenomenon Turner [1929, 3] has stated that " deficiency of nutriment will affect both seed weight and lint weight, so that obviously the relative change in their ratio is likely to be less than the relative change in the lint weight alone ".

*Some correlation coefficients for the Early Strain and their significance.**

Properties	Correlation coefficient
(1) The mean seed weight and the mean average fibre length .	r = +0·48
(2) The mean seed weight and the mean number of hairs per seed .	r = +0·33
(3) The mean lint weight and the mean fibre weight per unit length	r = +0·93
(4) The mean seed weight and the weight of a fibre of average length	r = +0·72

* It is well to state here that the number of pairs of readings for the sake of these calculations was as low as *seven*, this limitation being imposed by the number of seeds in a lock for which the measurements concerned were possible.

In order to test the significance of the above correlations it is necessary to calculate the probability that such a correlation should occur in a random sample in a non-correlated population. A significant correlation corresponds to a low value of this probability and *vice versa*. Using the tables given by Fisher [1930], it is found that in the present case (Table V) the correlation between the mean lint weight and the mean fibre weight per unit length is highly significant. There does not, however, seem to exist any correlation between the mean seed weight and either the mean average fibre length or the mean number of hairs per seed. The correlation between the mean seed weight and the weight of a fibre of average length is not significant.

TABLE V.

Application of Fisher's t. p. criterion to correlation coefficients from small samples.

Correlated properties	Correlation coefficient r	n (No. of pairs of observations minus 2).	$t = \frac{r}{\sqrt{1-r^2}} \times \sqrt{n}$	Probability, P	Remarks
(1) Mean seed weight and mean average fibre length	+0·48	5	1·224	0·28	Non-significant
(2) Mean seed weight and mean number of hairs per seed	+0·38	5	0·734	0·50	Ditto
(3) Mean lint weight and mean fibre weight per unit length	+0·93	5	5·657	0·0025	Significant
(4) Mean seed weight and weight of a fibre of average length	+0·72	5	2·320	0·07	Non-significant

CONCLUSIONS.

(I) The seed and the lint weights are, on the whole, correlated. The apical seeds give the lowest seed and lint weights. The seed and lint weights in the case of the basal seeds are also low. These conclusions agree with those of Ramanatha Iyer.

As evident from the experiments with the Early Strain.

(II) The apical seeds give (*i*) fibres of the least weight per unit length, (*ii*) the lowest ginning percentage, and (*iii*) the smallest number of hairs per seed.

(III) The fibre weight per unit length and the ginning percentage are high for the basal seeds.

(IV) Average fibre length does not seem to vary with the seed positions, although Ramanatha Iyer expects "differences in lint lengths of seeds according to their position in the lock".

(V) The present work is in accord with Turner's conclusions regarding the absence of correlation between the number of hairs per seed and either the ginning percentage or the seed weight.

The conclusions arrived at from the present experiments with regard to the effect of position particularly on the seed weight, the lint weight and the fibre weight per unit length have been tested by Fisher's method of analysis of variance. It is found that the effect of position is significant in all the cases (Table VI). This result definitely indicates that it is not enough to ascribe the differences in the values of any one of these characters for different positions of the seeds in a lock to random sampling alone, but that these differences must be due to some other cause.

TABLE VI.

*Effect of position on seed-weight, lint weight and fibre weight as tested by Fisher's method of analysis of variance.**

Cotton	Effect of position on	n_1	n_2	z	Value of z for P=0·01	Remarks
1. Early Strain	Seed weight	6	54	0·9092	0·5795	Significant
	Lint weight	6	54	0·9504	0·5795	Significant
	Fibre weight	6	24	0·9068	0·6496	Significant
2. P.—A. 4 F.	Seed weight	6	54	0·8508	0·5795	Significant
	Lint weight	6	54	0·5701(†)	0·5795	Doubtfully significant
3. P.—A. 289 F	Seed weight	6	54	0·7594	0·5795	Significant
	Lint weight	6	54	0·4721(†)	0·5795	Doubtfully significant
4. Early Strain 4 F and 289 F	Seed weight	6	180	1·0587	0·5152	Significant
	Lint weight	6	180	0·9634	0·5152	Significant

ACKNOWLEDGMENTS.

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* For application of the method of analysis of variance to the present data the author is indebted to Mr. S. S. Iyer, M.A.

† If the odds 1:20 (p=0·05) are regarded as sufficient for differentiation, the value of z, for $n_1=6$, and $n_2=54$, is 0·4135, consequently the variance may be regarded as significant.

A STATISTICAL NOTE ON THE ANALYSIS OF VARIANCE.

The mean seed weights and lint weights for the seven different positions of seed in the lock for each of the ten lots give in all 69 degrees of freedom for analysis in each of the three varieties of cotton, *viz.*, the Early Strain, P.-A. 4 F. and P.-A. 289 F. First, the variation in seed weight and lint weight with respect to the position of seed in the lock was studied separately for each of the varieties and the results are summarized in Table I below. There being only 5 lots available for the study of variation in fibre weight, the total number of degrees of freedom to be analysed in this case was only 34. These results are also given in Table I. The effect of position is significant in all the cases studied.

It will be noted that in the case of seed weight and lint weight random variation has been assigned only 54 degrees of freedom instead of 63, and for fibre weight only 24 instead of 28. This is due to the fact that 9 degrees of freedom in the former and 4 in the latter have been separated from the total for residual variance and ascribed to what has been called the variation between 'lots'. Even though the different lots had been chosen at random the analysis shows that there still remains a significant variation between the 'lots' (*c. f.*, variation between blocks in the case of a 'randomised blocks' field experiment). In fact, in the case of seed weight for both the Early Strain and 289 F. the effect of 'position is definitely marked by the variation between lots as is evident by comparing the results before removing the 9 degrees of freedom with those after the removal. For $n_1=6$ and $n_2=63$ the values of z in the two cases are 0·3999 and 0·3202 and are not significant, while as a matter of fact the effect of position is significant. In most of the remaining cases also the variation between 'lots' was quite significant. Therefore it is necessary to remove this spurious variation from the residual variance to make the comparisons between the different positions strictly valid.

A more comprehensive analysis was also made in the case of the mean seed and lint weights by taking all the three varieties together. The results are summarised in Table II. In both cases the effect of 'varieties' as well as that of 'position' is statistically significant. As can be expected the differential response of varieties with regard to position is quite insignificant.

Conclusion.

From the above analysis it is evident that in the case of all the three characters studied and in the case of all the three varieties of the Punjab-American cottons examined, the position of seed in the lock has a marked effect and the differences observed with regard to the position must be regarded as due to some other cause, than mere random sampling fluctuations.

TABLE I.

Significance of the seed weight, the lint weight and the fibre weight with regard to the position of a seed in a lock.

Character studied	Variety	n_1	n_2	z	(*) Value of z for P=0·01	Remarks
Seed weight	Early Strain	6	54	0·9092	0·5795	Significant
	P.-A. 4 F.	6	54	0·8508	0·5795	Significant
	P.-A. 289 F.	6	54	0·7594	0·5795	Significant
Lint weight	Early Strain	6	54	0·9504	0·5795	Significant
	P.-A. 4 F.	6	54	0·5701†	0·5795	Doubtfully significant
	P.-A. 289 F.	6	54	0·4721†	0·5795	Doubtfully significant
Fibre weight	Early Strain	6	24	0·9068	0·6496	Significant

TABLE II.

Significance of the seed weight and the lint weight with regard to varieties of cotton and to the position of a seed in a lock.

Character studied	Variety	Significance of	n_1	n_2	z	(*) Value of z for P=0·01	Remarks
Seed weight	Early Strain	1. Position	6	180	1·0587	0·5152	Significant
		2. Varieties	2	180	2·6257	0·7636	Significant
	P.-A. 4 F. and P.-A. 289 F.	3. Diff. response.	12	180	Negative	0·3908	Non-significant
		4. Lots	9	180	0·9706	0·4430	Significant
Lint weight	Early Strain	1. Position	6	180	0·9634	0·5152	Significant
		2. Varieties	2	180	1·9260	0·7636	Significant
	P.-A. 4 F. and P.-A. 289 F.	3. Diff. response.	12	180	Negative	0·3908	Non-significant
		4. Lots	9	180	0·5678	0·4430	Significant

* Values calculated from 'z' table in "Statistical Methods for Research Workers" by R. A. Fisher (1930).

† If we regard the odds of 1 : 20 (P=0·05) sufficient for differentiation, the value of z, for $n_1=6$, $n_2=54$, is 0·4135, hence the variance may be regarded as significant.

REFERENCES.

Afzal, M. (1930). *Mem. Dept. Agric. Ind., Bot. Ser.* 17, No. 4, 115.

Afzal, M. and Trought, T. (1932). "Motes in Cotton" (*in the press*) 1. Punjab-American Cottons.

Trought, T. (1931). *Ind. J. Agric. Sci.* 1, 335.

Balls, W. L. (1915). "The Development and Properties of Raw Cotton". A. and C. Black Ltd., London.

——— (1928). "Studies of Quality in Cotton", Ch. III., McMillian & Co., London.

Engledow, F. L., and Wadham, S. M. (1923). *J. Agric. Sci.* 13, 418, etc.

Fisher, R. A. (1930). "Statistical Methods for Research Workers" pages 139, 159 and 176.

Kearney, T. H. (1928). As reproduced in *Agric. J. Ind.*, 33, 290 from *J. Agric. Res.* 33, No. 8.

Ramanatha Iyer, V. (1929). Proc. Sixteenth, Ind. Sci. Congress (Madras) Third Circuit Agric. Section, P. 34.

———(1930). *Agric. J. Ind.* 15, 52.

Turner, A. J. (1929, 1). *J. Text. Inst.* 20, 9 or Tech. Bull. (Ind. Cent. Cotton Comm., Ser. B, No. 4) p. 5.

——— (1929, 2). *Ibid* p. 21.

——— (1929, 3). *Ibid* p. 24.

——— (1929, 4). *Ibid* p. 23.

——— (1929, 5). *Ibid* p. 1.

——— (1930). "Tech. Rep. on Standard Indian Cottons" (Ind. Cent. Cotton Comm. p. 18).

SOME PRELIMINARY STUDIES ON GRAM-BLIGHT WITH REFERENCE TO ITS CAUSE AND MODE OF PERENNATION.

BY

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(With Plates XLIX-LIII and two text-figs.)

Introduction.

The gram crop in the Attock and adjoining districts of Rawalpindi and Jhelum has been subject to the attack of the blight disease, which has seriously damaged the crop for a number of years. Next to wheat, gram (*Cicer arietinum* L.) is the most important *rabi* (spring crop) *barani* crop of the Attock district and occupies an area of about one lakh acres. In certain years when climatic conditions are favourable to the disease, the crop becomes almost totally destroyed in many places. Recently the disease has been observed in several other parts of the Province, *i.e.*, Gujrat, Gurdaspur and Lyallpur, although the damage done in these places is not serious, possibly due to the climatic conditions not being so favourable to the disease there as in the north.

The investigation of the disease was taken up by the senior author of the paper in 1926. Examination of samples of diseased plants collected from fields at Talagang (Attock district) revealed the presence of a fungus which on identification at Lyallpur and with the concurrence of the Imperial Mycologist, Imperial Institute of Agricultural Research, Pusa (Bihar) was considered to be *Mycosphaerella pinodes* (Berk and Blox) Stone= *Ascochyta pisi* Lib. A report of the investigations then made chiefly describing the symptoms and the nature of the damage was submitted to the Director of Agriculture, Punjab; also a popular note was published in the "Seasonal Note" of the Punjab Agricultural Department, Vol. III, No. 2, October, 1926.

Symptoms.—Symptoms of the gram-blight disease as studied on the specimens collected are as follows:—

All the above-ground parts are affected by the disease. Brown spots varying in size appear on branches, leaf stalks, and leaflets. On the stem the lesions are prominent and there may be several of them at intervals. Generally the spots encircle the stem completely and the parts of the plant above the lesions wilt and

droop down. In case where the spots are found at the base of a plant the whole of the plant withers. As a rule lesions are found on the terminal portions of shoots. In this respect gram-blight presents a striking contrast to the wilt disease of gram in which case the whole plant wilts invariably, due to the disease having affected the roots. The pods of diseased plants bear prominent concentric spots of dark brown colour. The characteristic feature of the spots is that they are dotted over with numerous dark bodies called pycnidia (Plate XLIX). When a section passing through a pycnidium is examined under the microscope, it looks spherical or pear-shaped, with an opening called the ostiole at the top and numerous oval or oblong single-celled pycnospores inside it.

A causal fungus.—Gram-blight has been also reported from France, Italy and Spain. The fungus responsible for this disease in these countries has been named *Phyllosticta rabiei* (Pass) Trotter. In the course of examination of the diseased specimens it was found that the characters of a fungus isolated for detailed study resembled closely those of *Phyllosticta rabiei* (Pass) Trotter. In order to determine if the specimens of gram-blight fungus as collected here in the Punjab are identical with it, actual specimens of *Phyllosticta rabiei* on gram with pods were obtained for study from Professor L. Petri, Director of the Royal Station of Vegetable Pathology at Rome.

A comparison of the local gram-blight fungus and *Phyllosticta rabiei* (Pass) Trotter is given below :—

Local gram blight fungus.—Pycnidia concentrically arranged on the pods, size : 163·75 ×148·20 microns. Pycnospores : hyaline, oval or oblong, unicellular without a median septum. Size : 10·75×5·1 microns.

Pycnospores of the local gram fungus were examined in all stages of development. Almost in all cases they were unicellular and unseptate. Rarely a bicellular spore was noticed.

Phyllosticta rabiei (Pass) Trotter.—Pycnidia concentrically arranged on the pods and leaves. Size, 181·64×166·20 microns. Pycnospores : hyaline, oval or oblong, unicellular with no median septum. Size : 11·0×5·20 microns (Plate L).

The above comparison of the characters of the two fungi clearly shows that they are closely related to each other.

A STUDY OF THE LOCAL GRAM FUNGUS AND *Phyllosticta rabiei* (PASS) TROTTER, ON CULTURE MEDIA.

The local gram fungus was isolated from lesions on plants obtained from Campbellpur in February, 1931, and was grown on pure cultures from single spores. A pure culture of *Phyllosticta rabiei* (Pass) Trotter was obtained from Schimmel-cultures, Baarn, Holland. Media used for the cultural study were (1) Oatmeal agar,



FIG. 2



FIG. 1

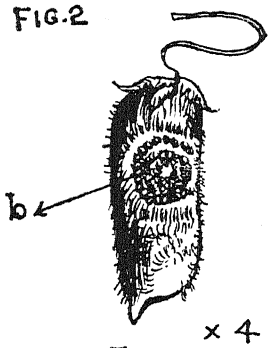


FIG. 4 × 4



FIG. 5 × 25

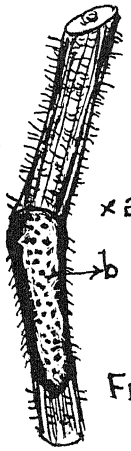
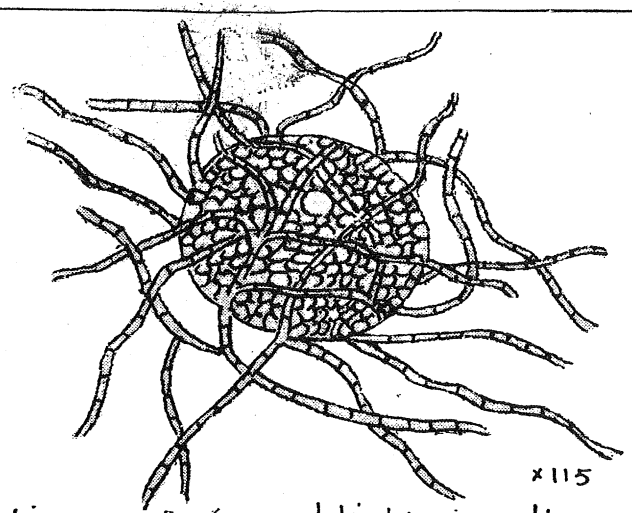


FIG. 3

(For explanation please see page 514.)

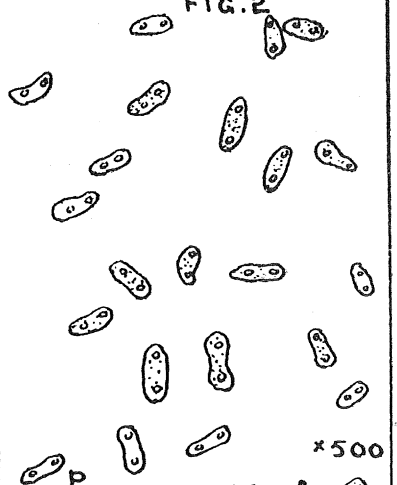
FIG. 1



×115

A pycnidium of gram blight in culture

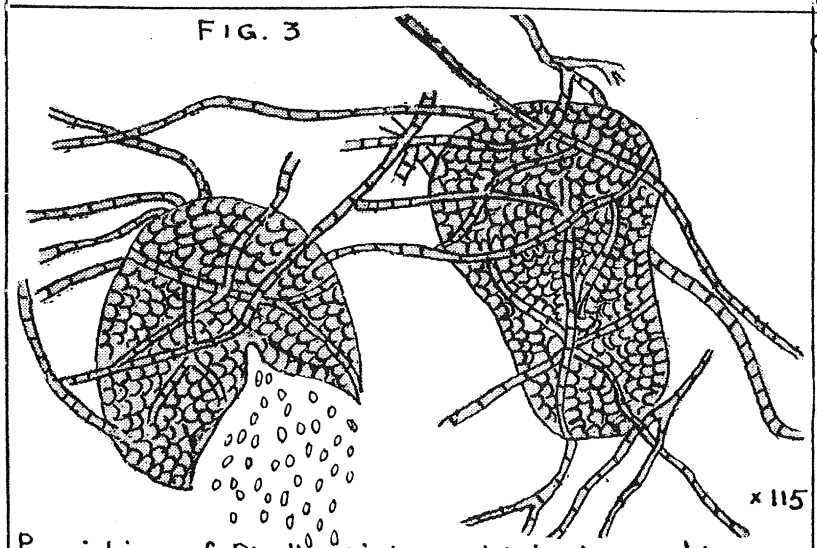
FIG. 2



×500

Pycnospores of gram blight in culture

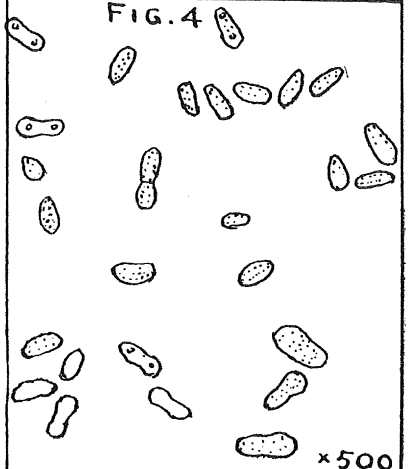
FIG. 3



×115

Pycnidia of Phyllosticta rabiei in culture

FIG. 4



×500

Pycnospores of Phyllosticta rabiei in culture

(2) Potato-dextrose agar, (3) Gram seed extract agar, (4) Richards' agar, (5) Nutrient glucose agar and (6) Gram-meal agar.

Four plates of each medium were inoculated on the 20th March, 1931, excepting gram-meal agar which was inoculated on 7th January, 1932, with the fungi referred to and were placed in the culture room the temperature of which varied from 70° 80° F. The following observations were made on the cultures :—

Colour, rate of radial growth, outline, margin, surface, relative amount and nature of aerial mycelium, zonation, sporulation and other outstanding features of the colonies.

Characters studied under these heads are given in Table I.

A study of the table and photographs of culture plates (Pl. LI) will show that the local gram fungus from Cambellpur and *Phyllosticta rabiei* (Pass) Trotter are very similar with regard to most of the cultural characters. *Phyllosticta rabiei* has, however, a sightly higher rate of growth (Figs. 1 and 2). Besides, there are also noticeable some other minor differences, but they should not alter the identical position of the two organisms. For example, on nutrient glucose agar colonies of the local gram fungus bear large pink masses of pycnospores exuded from pycnidia, while those of *Phyllosticta rabiei* are not so abundant. Another difference is that on oatmeal agar colonies of the local gram fungus are somewhat more elevated than those of *Phyllosticta rabiei*.

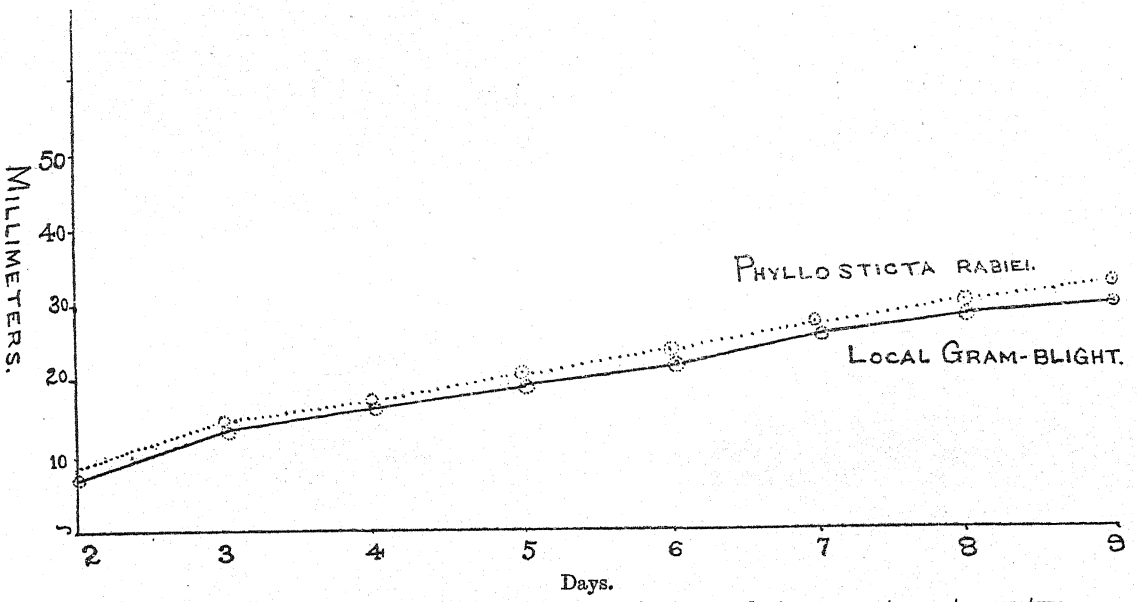


Fig. 1.—Rate of growth of local gram-blight and *Phyllosticta rabiei* on potato-dextrose agar at room temperature.

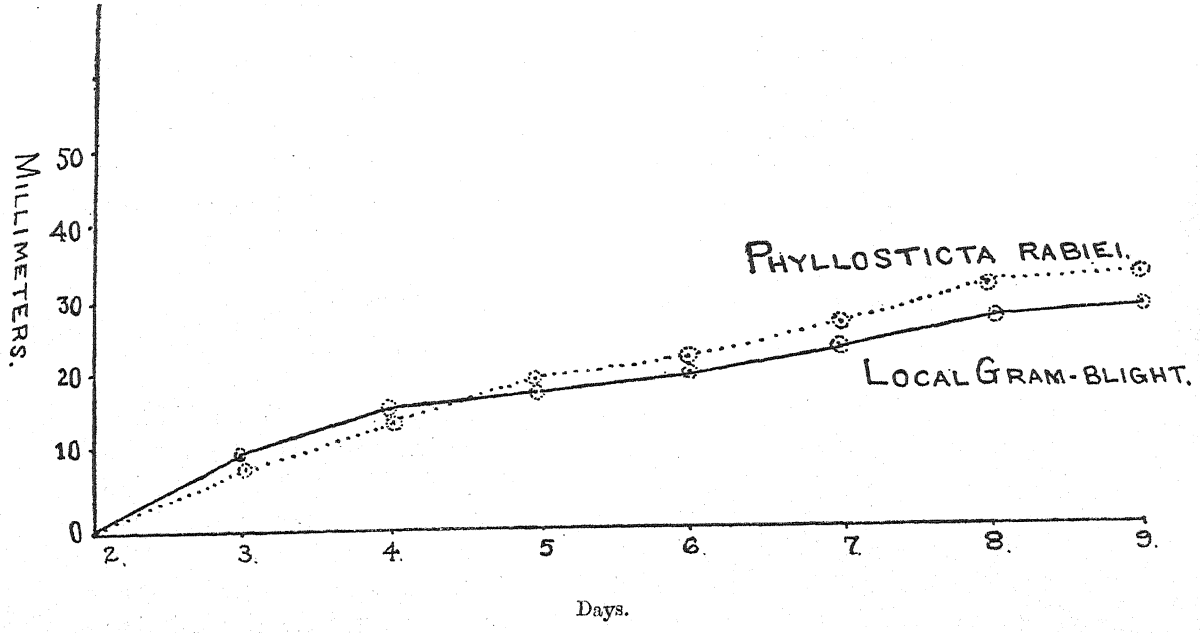


Fig. 2.—Rate of growth of local gram-blight and *Phyllosticta rabiei* on oatmeal agar at room temperature.

Chromogeny.—For the study of chromogeny sterilized rice culture tubes were inoculated on the 20th March, 1931 with the local gram fungus and *Phyllosticta rabiei*. The tubes are examined after 8 days. Both the fungi were found to be distinctly non-chromogenic.

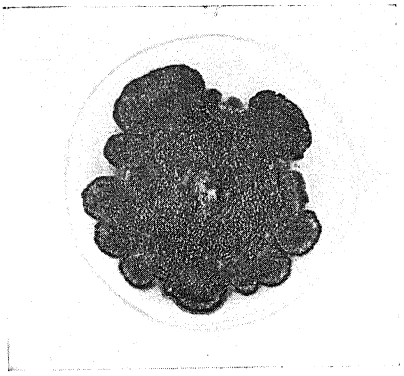


Fig. 1.

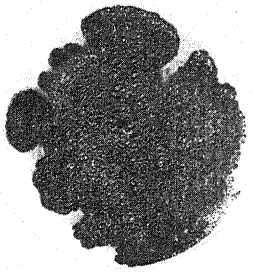


Fig. 2.

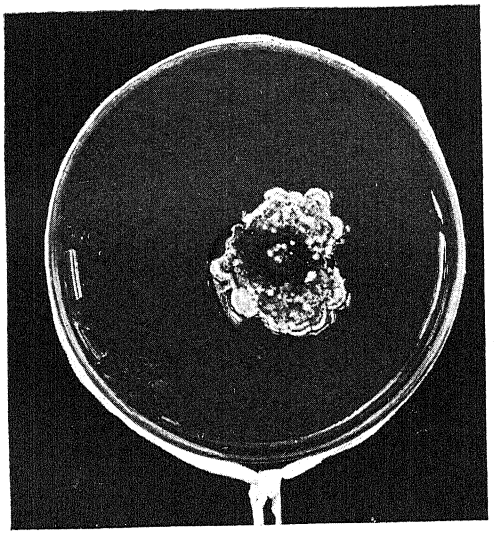


Fig. 3.

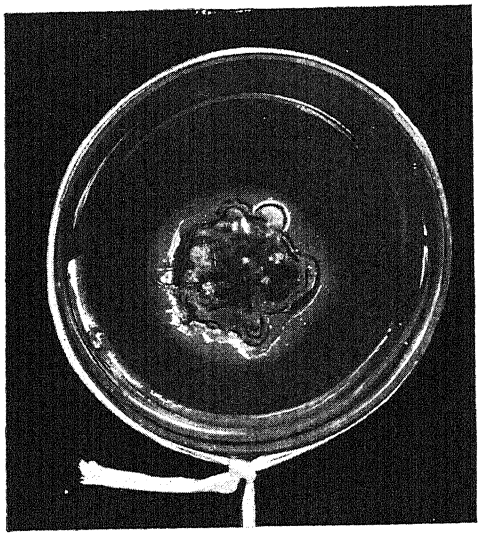


Fig. 4.

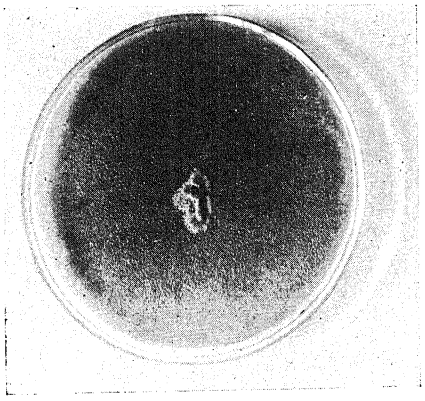


Fig. 5.

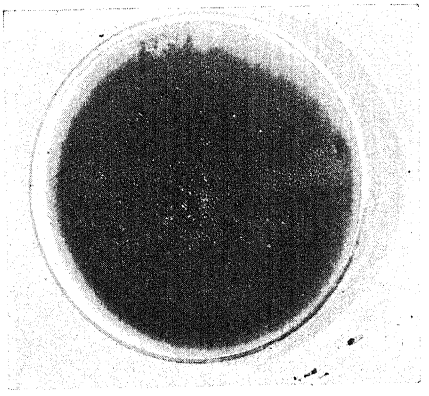


Fig. 6.

(For explanation please see page 514.)

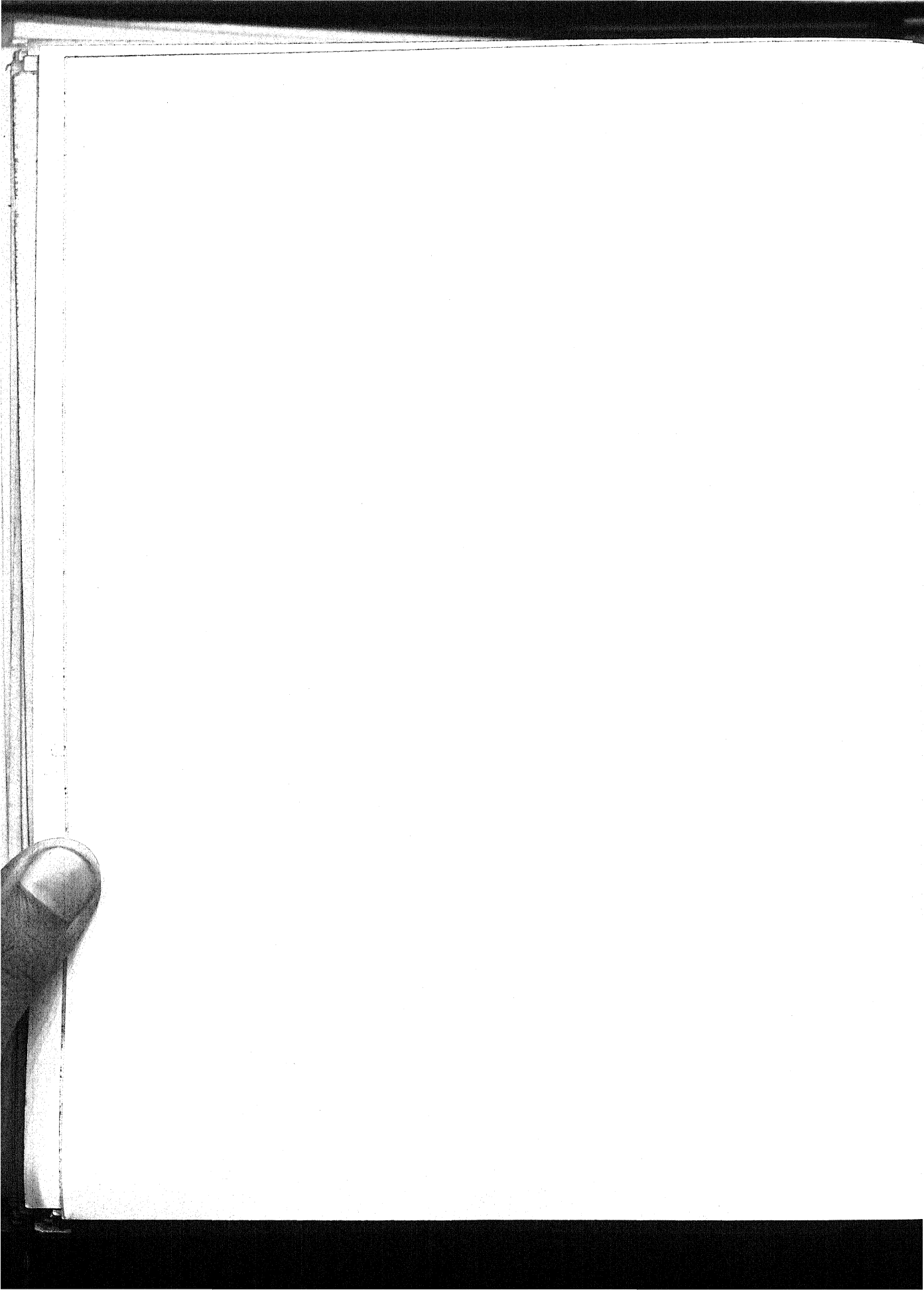


TABLE I.

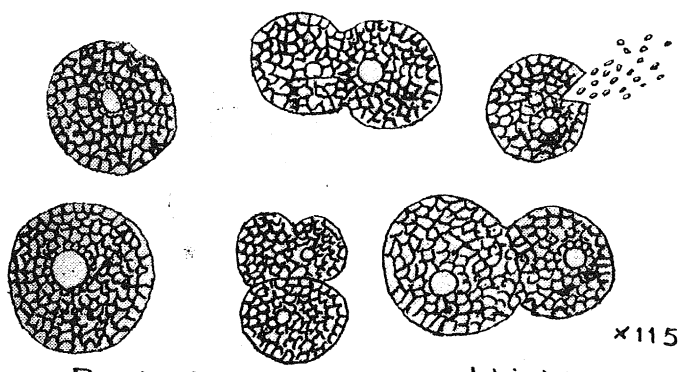
Summary of cultural characters of local gram-blight and Phyllosticta rabiei (*Pass*) *Trotter, on various culture media.*

Culture medium	Name of the fungus	Age of colony in days	Diameter of colony in mm.	Colour of culture	Outline of colony	Margin	Surface	Aerial mycelium	Zonation	Sporulation	REMARKS
Oatmeal agar	1. Local gram-blight (Pl. LI, fig. 1)	12	38	Almost black .	Irregular .	Very wavy	Smooth .	Almost absent	*Nil*	Copious and rapid	Grows very slowly, black pycnidia numerous and clearly visible
	2. *Phyllosticta rabiei* (Pass) Trotter (Pl. LI, fig. 2)	12	40	Black . .	Ditto .	Ditto	Do. .	Ditto	*Nil*	Ditto	Grows very slowly, black pycnidia numerous and clearly visible. Pink masses of pycnospores are copiously exuded
Potato-dextrose agar	1. Local gram-blight	9	28	Dirty white to Pallid Mouse gray in the centre and iron gray on the margin	Somewhat irregular	Wavy .	Uneven, thrown up into folds on the periphery	Strong short	*Nil*	Abundant	Very slow growing
	2. *Phyllosticta rabiei* (Pass) Trotter	9	32	Same but with a yellowish hue in the centre	Circular .	Somewhat wavy	Uneven, thrown up into folds on the periphery	Ditto	Slight	Abundant	Ditto
Gram extract agar	1. Local gram-blight	15	38	Gray . .	More or less circular	Somewhat wavy	Even . .	Poor short	*Nil*	Abundant and rapid	Very slow growing
	2. *Phyllosticta rabiei* (Pass) Trotter	15	42	Do. . .	Ditto .	Ditto .	Do. .	Ditto .	Fair .	Ditto .	Ditto.

TABLE I—*contd.*

Culture medium	Name of the fungus	Age of colony in days	Diameter of colony in mm.	Colour of culture	Outline of colony	Margin	Surface	Aerial mycelium	Zonation	Sporulation	REMARKS
Richards' agar	1. Local gram-blight (Pl. LI, fig. 3)	15	30	Grayish olive .	Very irregular	Very wavy	Very uneven	*Nil*	*Nil*	Scanty and late	Colonies much elevated, very rough and hard tending to produce cracks in the medium
	2. *Phyllosticta rabiei* (Pass) Trotter (Pl. LI, fig. 4)	15	33	Deep grayish olive	Ditto .	Ditto .	Ditto .	*Nil*	*Nil*	Ditto .	Colonies much elevated, very rough and hard producing cracks in the medium as above
Nutrient glucose agar	1. Local gram-blight	15	50	Deep grayish olive in the centre and pale Ecru Drab at the margin	More or less circular	Wavy, thin and mycelial	Uneven, elevated in the centre, gradually tapering towards the periphery, marked by deep radial depressions	Very poor confined to the centre only	Sharp	Copious and rapid	It exudes pink masses of pycnospores in abundance. Black pycnidia arranged in a concentrically zonated fashion
	2. *Phyllosticta rabiei* (Pass) Trotter	15	51	Same as above except that the colour is somewhat deeper	Ditto .	Very wavy, thin and mycelial	Exactly as above except that it is a little more elevated in the centre	Scanty confined to the centre only	Ditto	Ditto .	Same as above except that it exudes masses of pycnospores to a less extent
Gram meal agar	1. Local gram-blight (Pl. LI, fig. 5)	30	90	Almost black due to abundant black pycnidia	Circular .	Entire thin	Even . .	*Nil*	*Nil*	Copious and rapid	
	2. *Phyllosticta rabiei* (Pass) Trotter (Plate LI, fig. 6)	30	92	Almost the same as above	Ditto .	Ditto	Do. . .	*Nil*	*Nil*	Ditto	

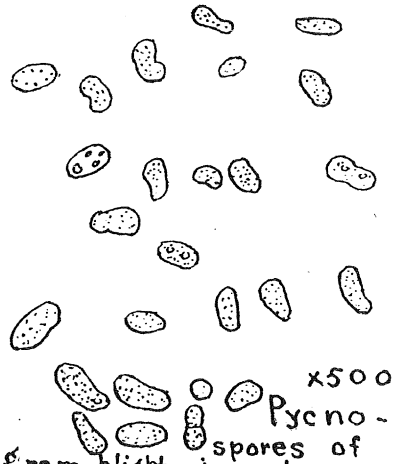
FIG. 1



×115

Pynidia of gram blight in nature

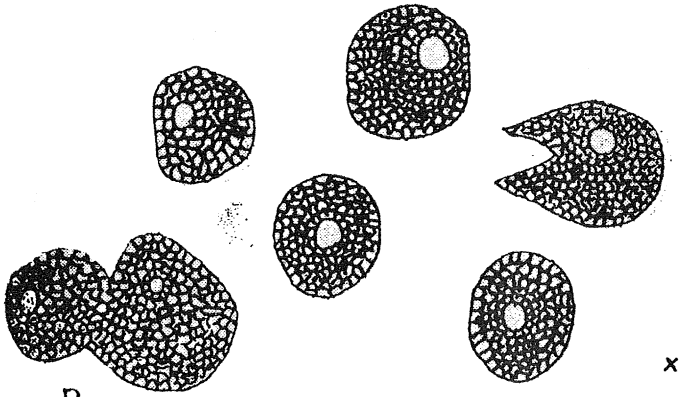
FIG. 2



×500

Pycno-spores of gram blight in nature

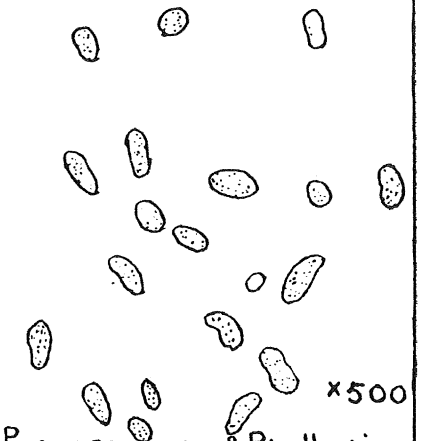
FIG. 3



× 115

Pycnidia of Phyllosticta rabiei in nature

FIG. 4



×500

Pycnospores of Phyllosticta rabiei in nature

MICROSCOPIC CHARACTERS OF THE FUNGI ON OATMEAL AGAR.

Local gram fungus.—Pycnidia : round, dark olive buff in colour with a peridium of polygonal cells, covered and surrounded by thick, dark, septate hyphae. Ostioles common. Size : 185×155 microns.

Pycnospores : hyaline, unicellular, oval or oblong, with rounded ends and two tiny oil globules near the extremities. In rare cases a faint median septum is observed. Size : 10·77×4·0 microns.

Aerial mycelium : 6·8 microns wide, hyaline and septate, with oil globules.

Phyllosticta rabiei (*Pass*) *Trotter.*—Pycnidia : round, oval or somewhat irregular, dark olive buff in colour, covered and surrounded by thick, dark, septate hyphae. Size : 200×167 microns. Ostioles visible in a less number of cases.

Pycnospores : hyaline, unicellular, oval or oblong, with rounded ends and two tiny oil globules near the extremities. One to two per cent. pycnospores showed a faint median septum. Size : 10·51×4·02 microns.

Aerial mycelium : hyaline and septate, 6·55 microns wide, with numerous oil globules (Pl. LII).

From the observations given above, it is further established that there is a close relationship between the local gram-blight organism and *Pyllosticta rabiei* (Pass) Trotter.

As mentioned above the pycnidia of the local gram-blight fungus are, however, somewhat smaller than those of *Phyllosticta rabiei*. The slight differences mentioned are, however, negligible and should not affect the identical nature of the two organisms.

The authors are indebted to Dr. W. McRae, D.Sc., Imperial Mycologist, Imperial Institute of Agricultural Research, Pusa (Bihar), for a biometrical analysis of the measurements of pycnospores of the two fungi from the data supplied to him. His note is reproduced below :—

....	Range	Mean	Length		Breadth	
			Standard deviation	Probable error	Standard deviation	Probable error
Phyllosticta rabiei (Nature) . .	3·9-17·5×3·9-5·8	11·1×5·3	2·3	·15	2·0	·13
Ditto Oat agar . .	5·8-13·7×2·9-5·8	10·7×4·0	2·7	·18	·5	·03
Gram-blight (Nature) . . .	3-17·5×2·5-7·8	10·7×5	2·8	·19	1·4	·09
Ditto Oat agar . . .	3·4-17·5×3·0-6·8	11 ×4	2·3	·15	·9	·04

The differences between the constants for *Phyllosticta rabiei* and "gram-blight" are not statistically significant.

According to these measurements then there is no difference between "gram-blight" and "*Phyllosticta rabiei*".

In addition to oatmeal agar culture the study of the pycnospores of the two fungi was made on four other culture media, *i.e.*, Richards' agar, gram extract agar, gram-meal agar and pea-soup agar. The results are given in Table II. It will be seen from the table that the two fungi are alike with regard to the characters and dimensions of spores. The identical form of the two fungi in relation to spores is established by these studies. If there is variation in size in the case of one on a culture medium, the other fungus also shows the same. The size of the spores of both the fungi has increased on gram-meal agar and pea-soup agar.

TABLE II.

Description of the pycnospores of local gram-blight and Phyllosticta rabiei (*Pass*) *Trotter, on various culture media.*

Serial No.	Name of the fungus	Richards' agar	Gram-extract agar	Gram-meal agar	Pea-soap agar
1	Local gram-blight	Hyaline, unicellular, oval to oblong, measuring 10·05×4 microns	Hyaline, unicellular, oval to oblong, protoplasmic contents granular and measuring 10·0×4·5 microns	Hyaline, unicellular, oval to oblong, measuring 11·04×4·30 microns	Hyaline, unicellular, oval to oblong, measuring 11×4·6 microns
2	*Phyllosticta rabiei* (Pass) Trotter	Hyaline, unicellular, oval to oblong, measuring 10·5×4·10 microns	Hyaline, unicellular, oval to oblong; many with protoplasmic contents granular near the ends, and measuring 10·02×4·03 microns	Hyaline, unicellular, oval to oblong measuring 10·72×4·16 microns	Hyaline, unicellular, oval to oblong, with 2 large vacuoles near the ends, and measuring 11·05×4·25 microns

GERMINATION STUDIES.

Pycnospores from 15 days old cultures of the local gram-blight fungus and *Phyllosticta rabiei* (Pass) Trotter, on gram leaf extract agar, were sown in drops of the following solutions on clean flamed glass slides :—

(*a*) Sterilized distilled water,

(*b*) 5 per cent. glucose solution,

(*c*) Gram seed extract, and

(*d*) Gram leaf extract.

The slides were arranged on clean glass rakes placed in moist chambers. The moist chambers were placed in incubators set at the following temperatures :—

(1) 8°—10°C.
(2) 15°C.
(3) 20°C.
(4) 25°C. and
(5) 32°C.

The slides were examined after 16 hours. No spores of any of the two fungi were found to have germinated in sterilized distilled water and 5 per cent. glucose solution at any of the temperatures given above. On the other hand nearly all the pycnospores of both the fungi had germinated in gram seed and gram leaf extracts at 15°C., 20°C. and 25°C.

Germination did not take place at 8°—10°C. and 32°C. even in gram seed and gram leaf extracts at the end of even three days.

Pycnospores of both the fungi germinate in exactly the same manner. Germination begins after about 8 hours and more than 95 per cent. pycnospores had germinated within 16 hours. Germ tube comes out generally from one end of the pycnospore. However, spores are observed producing germ tubes both ways and even from their centre. The germ tubes are at first continuous, but subsequently form septa and branch. The germ tubes of neighbouring pycnospores anastomose.

Inoculation experiments.

Six potted gram plants were inoculated on the 20th April, 1931, by atomising them with a suspension of pycnospores from a pure culture of *Phyllosticta rabiei* (Pass) Trotter. Six similar potted plants were kept as control. The plants were thoroughly washed with sterilized water before inoculating them. Inoculations were made late in the afternoon and the inoculated plants were covered with disinfected bell-jars for 72 hours. After 4 days, symptoms of infection were noticed on the inoculated plants. Lesions first appeared on the leaves and in severe cases pycnidia were also formed on some spots. Stems and branches also bore diseased spots and had turned brown. Affected leaves and branches drooped and ultimately dried up. The symptoms produced by *Phyllosticta rabiei* were similar to those of the local gram-blight fungus.

With a view to confirming the results of inoculation tests made in 1931 and extending the work for obtaining conclusive data, further experiments were conducted in February, 1932, as described below :—

On the 18th February, 1932, three sets each of 5 healthy potted gram plants of the common type were selected and washed with sterilized water. Five plants

were inoculated by each of the two fungi, *i.e.*, the local gram-blight fungus and *Phyllosticta rabiei* (Pass) Trotter by the same method as already described, and employed last year excepting that the plants were placed in disinfected glass chambers. The spore suspensions of the two fungi were taken from pure cultures on gram-meal agar. Five plants were left uninoculated to serve as control. After 4 days, *i.e.*, on the 22nd February, 1932, young terminal shoots of the inoculated plants began to show signs of drooping and withering. A few days later, typical, dark brown elongated lesions appeared on the branches. Dark circular spots with pycnidia concentrically arranged appeared on the pods in large numbers. Pycnidia when examined in water under the microscope yielded numerous hyaline, unicellular, oval or oblong pycnospores identical with those in the suspension used for inoculation. Within 10 days the leaves and branches of the inoculated plants were almost killed.

The control plants remained healthy and green (Plate LIII, figs. 1, 2 and 3).

The inoculation experiments were repeated after a week and were successful in producing the disease as before.

Another inoculation experiment was made on the 6th March, 1932. In this case only detached green gram pods were taken and surface sterilized with 0·1 per cent. mercuric chloride solution for about a minute and then were thoroughly rinsed with sterilized water with a view to freeing them of organisms present on the surface. By means of a sterilized platinum needle, mycelium and pycnidia containing pycnospores of *Phyllosticta rabiei* (Pass) Trotter and the local gram-blight fungus obtained from culture were each placed on one dozen pods. A third set of 12 pods was left uninoculated to serve as control. Both the inoculated and uninoculated sets of pods were placed in three separate sterilized petri dishes containing moist sterile filter papers and covered with lids. The petri dishes were placed in moist chambers at the room temperature of 70°—75°F. After 10 days the pods were examined on the 15th March, 1932. The pods inoculated with both the fungi produced typical, circular dark brown spots bearing pycnidia. Moreover, the seeds within the pods were, on examination, found to have dark spots on the testa. In some seeds the entire surface had turned black.

The uninoculated pods remained green and free from the disease.

It is interesting to note that in one case of inoculation with the local gram fungus, the seed had germinated during the experiment. Dark spots had appeared on the radicle. Sections of the diseased portion were cut and examined. Many hyphae inter and intra-cellular were found.

These inoculation experiments prove conclusively that *Phyllosticta rabiei* (Pass) Trotter causes the same blight disease as the local gram fungus, under investigation, does.

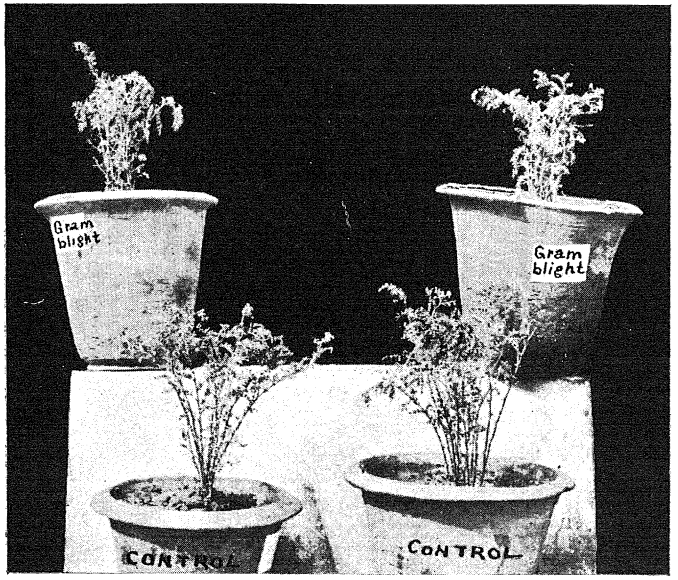


Fig. 1.

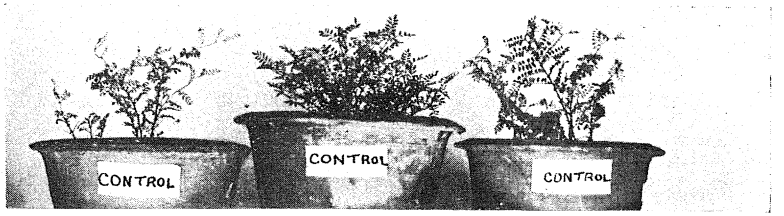


Fig. 2.

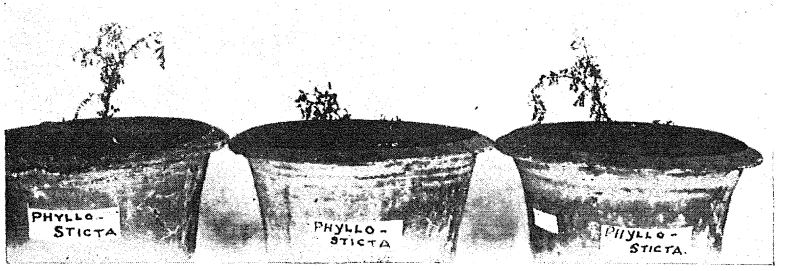


Fig. 3.

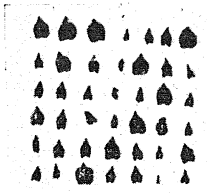


Fig. 4.

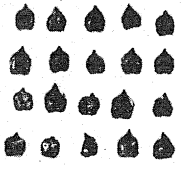
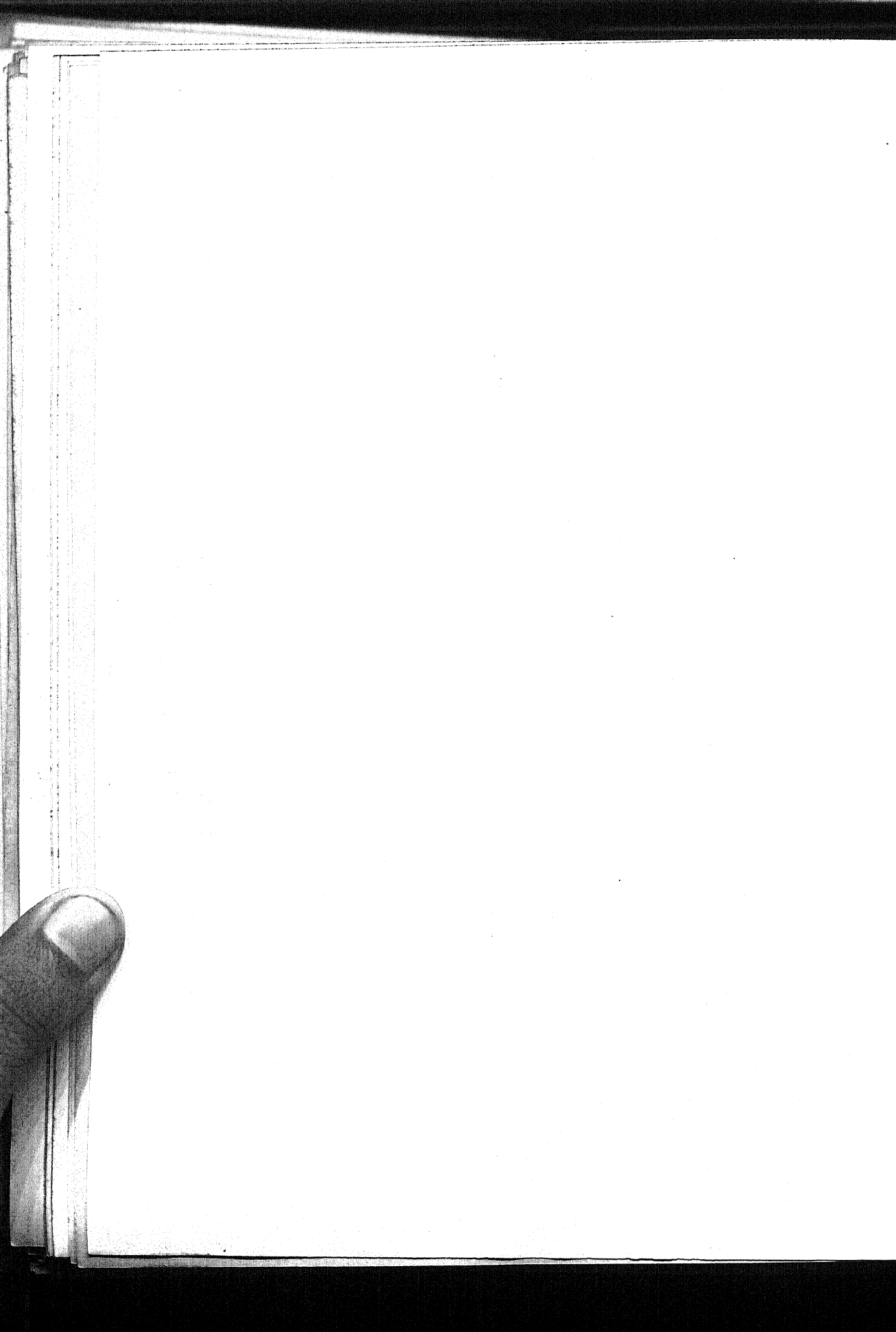


Fig. 5.

(For explanation please see page 515.)



Effect of the disease on gram seeds.

Seeds also have been found affected in pods by the disease. It appears that the fungus penetrates from the ovary wall into the testa of the seed at the point of contact. As badly diseased plants wither before maturity the pods also dry up, and the seed remains small and immature.

Dark spots characteristic of the disease have been noticed on the testa of the seeds from affected pods. Cotyledons of these seeds also show some spots. Spots on the ovary wall, testa and the cotyledons correspond in their position and underlie one another in this order. Some seeds are much shrivelled and are altogether useless for any purpose (Pl. LIII, figs. 4 and 5). These seeds have not been found to germinate. There are, however, found always a few plants in the field on which pods with diseased spots are present, but no obvious damage seems to have been caused to the seed, as they are fully developed like normal seeds and germinate quite well. These seeds also, however, bear diseased spots. Sections of the testa and cotyledons have shown that there are hyphae in them. The hyphae are branched, hyaline and septate with oil globules. They are inter as well as intracellular.

A comparison of seeds from (1) healthy pods, (2) diseased pods, regarding absolute weight and germination capacity is given below :—

Kind of seed	Absolute weight per 100 seeds	Percentage germination capacity
1. Healthy seed	12·57 grms.	99·3
2. Diseased ,, ...	3·948 ,,	44·6

Mode of infection and perennation.

As mentioned above, the blight fungus forms diseased spots on the pods as well as on the testa and cotyledons. To investigate if the seeds carry the fungus, the infected seeds after surface sterilization were placed in plain agar slants and were placed in a chamber at the room temperature of 72°—77°F. on 14th April, 1932. On the 4th day a mycelial growth appeared on the slants and produced numerous pycnidia containing hyaline unicellular spores within a week. Pycnidia were also observed in abundance on the testa of the seeds. Some of the seeds had germinated on the slants. A diseased spot 5 days after germination appeared on the plumule just below the tip. The lesion was dark brown, elongated, and was covered with

numerous pycnidia, typical of the disease. These observations show that the seed carries the fungus. It is probable that primarily the disease is produced in the field by sowing diseased seeds. In localities where the disease has been prevalent, seed from the local diseased crop is generally used for sowing purposes. There is, no doubt, that most of the seed is infected. As stated above about 45 per cent. of the seeds harvested from the diseased crop are able to germinate. Some of these viable seeds are infected. The plants to which they give rise will bear the disease in the first instance. Lesions are formed on the branches and pycnidia and spores are produced in large numbers. The spores are liberated from the pycnidia and are disseminated by wind. If the conditions are favourable such as moist weather and strong winds, the spores that are carried to other plants in the fields stick to their surface, germinate and cause infection. In this way, secondary infection is brought about very rapidly. The disease first appears on a few plants, in the beginning or the middle of January but continues to spread in the fields involving more and more plants gradually. Generally it is found in patches which get enlarged as the disease progresses. Under conditions favourable for the dissemination and germination of spores, which usually prevail in the beginning of March, when flowering and fruit-setting commences, the attack of the fungus is very severe and takes the form of an epidemic. It has been observed that flourishing fields of the crop are scorched and altogether killed by the disease within a period of one to two weeks. Most of the disease is caused by secondary infection.

The following experiments were carried out in 1930-31 for the study of the mode of perennation of the fungus :—

1. Botanical Farm, Lyallpur.

Field experiments.—Seed obtained from a severely infected crop was obtained from Rawalpindi. It was sown in 1930 at Lyallpur with healthy gram type No. 7 in alternate plots measuring 45 ft. × 8 ft. The crop raised from the diseased Rawalpindi seed was found to be affected by the disease in the last week of March. About 56 per cent. of the plants were attacked. The disease was apparent mostly on the pods. The crop in the adjacent plots of healthy type No. 7 was also somewhat diseased, but the attack was only 2·7 per cent. This was due to secondary infection from the diseased plots. Gram had never been sown on this piece of land before. There was no gram-blight disease in any of the gram plots in the Botanical area. The results show that the disease was carried by the use of seed which was taken from the diseased crop. The possibility of soil being a contributory cause is obviously excluded. In October, 1931, gram seed obtained from Ferozepur where the disease is not found was sown on the plot which had borne a diseased crop of 56 per cent. intensity in the previous season. The crop pro-

duced was free from the disease. This shows that no effect of the diseased crop was left in the soil from the previous year.

Pot experiments.—(*a*) Fifty earthen pots were filled with soil obtained from a field at Campbellpur, in which gram-blight had appeared in a severe form in March, 1930. If any infection remains in the soil it must have been present in the soil put into the pots. Healthy gram type No. 7 was sown in these pots. There were 4 plants in each pot. They were carefully examined periodically throughout the season. There was no sign of the disease on any of them.

(*b*) A second set of pot experiments was carried out similarly by using Lyallpur soil and seed from diseased crop, obtained from Rawalpindi. The soil used was taken from a field where no gram was ever grown. In this case almost all the plants were attacked by the disease.

These two pot experiments corroborate the results of the field experiments.

2. Agricultural Farm, Rawalpindi.

In 1929-30 the gram crop at the farm covering about 10 acres was badly attacked by the gram-blight disease. Half an acre of this was sown each with healthy seed of gram types Nos. 7 and 15 respectively in October, 1930. The crop raised was absolutely free from the disease.

3. Agricultural Farm, Campbellpur.

(*a*) Two plots, 2 acres each, of the land which had in the previous year borne a diseased crop, were each sown with Punjab gram types 7 and 15 respectively. Careful observations were made on the crop throughout the season and no trace of the disease was found. There were several gram fields of farmers in the vicinity of the farm which were attacked by the disease. But as the climatic conditions were not suitable for secondary infection, the disease was not carried to the experimental crop on the farm.

(*b*) In this connection reference may be made to another small trial made on a zamindar's field. In this case diseased seed was sown on a half acre plot. The crop was found to be affected to the extent of 5·7 per cent.

(*c*) The results of experiments reported above undoubtedly show that soil does not harbour the disease and that the seed is the exclusive source of the disease in the primary stages. To further confirm this, a great deal of diseased parts of gram plants was buried in a portion of a field at the farm at a depth of about 3 in. in April, 1931, soon after harvest. The plot was left fallow until the next sowing season of the crop. The object was to add a big dose of infected material to the soil and give an opportunity to the fungus for causing the disease in the crop raised on it. In October, 1931, healthy seed of gram type 7 was sown

there. In March, 1932, when the disease broke out all over zamindars' gram fields in the vicinity, the crop remained free from the disease. Subsequently towards the end of the season there were some traces of the disease noticeable on the terminal shoots. This slight attack had been caused by secondary infection. There were wheat fields around this crop that worked as barriers and somewhat prevented the spores from reaching this plot though not completely. The experiment, however, conclusively, like other experiments described above, shows that soil plays no part in the carrying of the disease to the next season. No doubt usually considerable amount of diseased material consisting of branches, leaflets, and pods bearing the fungus is added to the fields from the crop, but it appears certain that during the heat of intense summer extending over 6 months, *i.e.*, April to September, the fungus cannot remain alive. Its spores and hyphae are killed and do not seem to over-summer to cause the disease.

Suggestions for control of the disease.

Experiments reported in the paper clearly indicate that seed harbours the fungus and the disease is produced by the use of infected seed for sowing purposes. The soil, as inferred from the experiments so far carried out, is not responsible for causing the disease. Therefore for control of the disease, seed from localities where the disease is prevalent should not be sown. Seed should be obtained from those places where the disease does not occur. In view of the fact that secondary infection brings about a large percentage of the disease, the presence of any crop raised from diseased seed in the vicinity of that produced from healthy seed would be dangerous as it would provide a source of infection. Consequently it seems necessary that the seed of localities where the disease is prevalent should be altogether replaced by healthy seed so that a diseased-free crop is raised *en bloc*.

Systematic position.

The systematic position of the gram-blight fungus and *Phyllosticta rabiei* (Pass) Trotter according to F. L. Stevens, *vide* his book "The fungi which cause plant disease" (pages 479-81), would be as follows :—

Fungi Imperfecti, Order=Sphaeropsidales, Family=Sphaerioidaceae, Section=Amerosporae, Sub-section=Hyalosporae, Genus=Phyllosticta.

But in this connection the writers have further to remark as below :—

The specimens of *Phyllosticta rabiei* (Pass) Trotter on gram plants received from Professor Trotter as well as the plants inoculated from cultures of the fungus obtained from Schimmelcultures, Baarn, Holland clearly bear lesions of the disease on all parts, *i.e.*, branches, leaflets and pods. In this respect the character of the

fungus differs from the description of the Phyllosticta genus as given by F. L. Stevens on page 484 of his book " Fungi that cause plant disease " where he states that it is foliicolous. If this character is strictly observed, the fungus on gram as investigated by the authors as well as that of Professor Trotter cannot be placed under Phyliosticta. One would be inclined to call it an Ascochyta which attacks all parts of an affected plant, but in view of the fact that the local gram fungus described in the paper has continuous or single-celled spores, it must be different from Ascochyta. The situation is that the gram-blight fungus shares the characters of both Phyllosticta and Ascochyta. Its exact systematic position has, therefore, to be finally settled. In this connection one's attention is bound to be directed to a third genus namely Phoma that might also be considered for defining the exact nomenclature of the fungus. Spores of Phoma are single-celled and are below 15 microns in length, but it does not attack leaves. On account of these differences, although minor, between the gram-blight fungus and the other genera referred to, its position requires to be cleared.

It might be mentioned that cultural studies, which the authors have been carrying on, have shown that the gram-blight fungus as it occurs in North Punjab consists of a large number of physiological forms. About a dozen distinct strains have been isolated.

Some of the work reported in the paper was started by late Dr. Kripa Ram Mohendra, Ph.D., Mycologist, Punjab, but it was interrupted by his untimely and sad death on 13th October, 1930.

Further work on the investigation on the gram-blight disease is in progress.

Summary.

1. The gram-blight disease has been found to cause serious damage to the gram crop in the North Punjab. A fungus isolated from the diseased gram plants has been studied, in regard to its morphological and cultural characters. It has also been compared with *Phyllosticta rabiei* (Pass) Trotter.

2. Symptoms of the disease as it occurs on branches, leaflets, pods and seeds are described.

3. The local gram-blight fungus and *Phyllosticta rabiei* (Pass) Trotter have been found to be identical.

4. Germination studies show that the spores germinate after 8 hours in gram seed and leaf extracts. Within 16 hours about 95 per cent. of the pycnospores complete their germination.

5. Inoculation experiments with gram-blight and *Phyllosticta rabiei* (Pass) Trotter have been made on plants grown in pots. The disease appeared on all the

inoculated plants after 4 days. These experiments establish that *Phyllosticta rabiei* is a cause of the gram disease in the North Punjab.

6. Spots of the disease are commonly found on the surface of the seeds. Sections of the testa and cotyledons have shown hyphae of the fungus in abundance.

7. Observations and experiments have shown that the disease is seed-borne.

REFERENCES.

Butler, E. J. (1918). "Fungi and Disease in plants", p. 268 and 269.

Chippindale, H. G. (1929). The development in culture of Ascochyta Gossypii Syd. British Mycological Society Transactions XIV.

Curtis, K. M. (1923). Ascochyta Pisi on Lupin. *Rev. of Applied Mycology*, **2**, 504.

Grace, G. Gilchrist (1926). *Phytopathology* **16**, 269.

Jones, L. K. (1927). *New York State Agric. Expt. Station, Geneva, N. Y. Bulletin* No. 547.

Jones, F. R. and Linford, M.B. (1925). *Research Bulletin* 64.

Labrousse, F. (1930). L'anthracnose du Pois-Ciche (Cicer arietinum) Extrait de la Revue de Pathologie Vegetale et d'Entomologie Agricole XVII, 4.

————(1932). L'anthracnose du Pois-Chiche. (Anthracnose of Chick Pea) *Review of Applied Mycology*, **11**, 150.

Luthra, Jai Chand (1926). 'A gram disease', *Seasonal Notes*, **3**, No. 2.

Ridgway, R. (1912). Colour Standards and Nomenclature.

Sprague, R. (1930). *Phytopathology* **20**, No. 7.

Stevens, F.L. (1913). "The fungi which cause plant disease."

EXPLANATION OF PLATES.

Plate XLIX

Fig. 1.—A branch of gram plant (*Cicer arietinum*) affected with gram-blight, showing (*a*) diseased spots.

Fig. 2.—Pods showing (*b*) pycnidia concentrically arranged.

Fig. 3.—A portion of stalk showing a lesion bearing (*b*) pycnidia.

Fig. 4.—A pod magnified 4 times showing (*b*) pycnidia.

Fig. 5.—A leaflet showing (*b*) pycnidia concentrically arranged.

Plate L

Fig. 1.—Pycnidia of gram-blight on gram leaves showing round ostioles and peridia of polygonal cells × 115.

Fig. 2.—Unicellular pycnospores of gram-blight on gram leaves × 500.

Fig. 3.—Pycnidia of *Phyllosticta rabiei* (Pass) Trotter on gram leaves showing round ostioles and peridia of polygonal cells × 115.

Fig. 4.—Unicellular pycnospores of *Phyllosticta rabiei* (Pass) Trotter on gram leaves × 500.

Plate LI

Fig. 1.—4 weeks old culture of local gram-blight on oatmeal agar.

Fig. 2.—4 weeks old culture of *Phyllosticta rabiei* (Pass) Trotter on oatmeal agar.

Fig. 3.—15 days old culture of local gram-blight on Richards' agar.

Fig. 4.—15 days old culture of *Phyllosticta rabiei* (Pass) Trotter on Richards' agar.

Fig. 5.—30 days old culture of local gram-blight on gram-meal agar.

Fig. 6.—30 days old culture of *Phyllosticta rabiei* (Pass) Trotter on gram-meal agar.

Plate LII

Fig. 1.—A Pycnidium of local gram-blight in culture on oatmeal agar, showing a round ostiole, a peridium of polygonal cells and surrounded and traversed by dark septate hyphae × 115.

Fig. 2.—Unicellular pycnospores of local gram-blight in culture on oatmeal agar × 500.

Fig. 3.—Pycnidia of *Phyllosticta rabiei* (Pass) Trotter on oatmeal agar with peridia of polygonal cells and surrounded and traversed by dark septate hyphae × 115.

Fig. 4.—Unicellular pycnospores of *Phyllosticta rabiei* (Pass) Trotter in culture on oatmeal agar × 500.

Plate LIII— Photographs showing :—

Fig. 1.—Potted gram plants killed by local gram-blight fungus after inoculation ; control plants.

Fig. 2.—Control plants.

Fig. 3.—Potted gram plants killed by *Phyllosticta rabiei* (Pass) Trotter, after inoculation.

Fig. 4.—Gram seeds from a crop affected by local gram-blight in March, 1932.

Fig. 5.—Gram seeds from a healthy crop.

MECHANICAL ANALYSIS OF LATERITIC SOILS.

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(With one text-fig.)

In recent years several methods of mechanical analysis have been developed to suit a large variety of soils but none of them are yet known to suit lateritic soils [Keen, 1931]. The value of mechanical analysis as an aid to the description and classification of soils have been fully discussed elsewhere [Olmstead, Alexander and Middleton, 1930; Rep. A. E. A., 1926]. The importance of finding a method for lateritic soils is therefore obvious specially for India where there are vast tracts of culturable land belonging to this type. The present investigation was therefore undertaken to test the suitability of some of the existing methods to laterites and to suggest modifications if necessary.

Of the existing methods, the one known as the International A method which has practically the same essential features as the revised British Official method [1928], has been adopted very widely. The dispersion of soil in this method is effected by a preliminary treatment with hydrogen peroxide to remove organic matter, followed by an acid treatment to destroy carbonates and finally by shaking a suspension of the treated soil made alkaline by ammonia. Since tropical soils contain generally little organic matter, the peroxide treatment was found unnecessary for such soils [Charlton, 1927; Puri and Bhailal, 1928]. Objection to the acid treatment has also been raised by Mattson [1927], and by Puri [1929] who advocates the use of NaCl in place of HCl, the final dispersion being made with a little NaOH. Similarly Joseph [1929] obtained the best result with heavy Sudan soils by using Na_2CO_3 as the dispersing agent without the previous acid treatment.* The clay in the soil in either method is thus converted into sodium clay which both these workers claim and rightly so, [Mattson, 1927] to be more easily dispersible than ammonium clay. Accordingly the International method was revised by Robinson [1931] who employed NaOH in place of ammonia.

* With certain Sudan soils which were found difficult to disperse a preliminary leaching with HCl proved satisfactory.

As regards the division of soil into fractions the Atterburg scale has now been universally adopted, which is as follows :—

Coarse sand 2·0 mm. to 0·2 mm. Fine sand 0·2 mm. to 0·02 mm.

Silt 0·02 mm. to 0·002 mm. and clay below 0·002 mm.

This scale in grouping the fractions was also adopted in this laboratory.

In the International and the Puri methods the pipette technique is employed to obtain the silt and clay fractions. In Joseph's method, which will be termed in this paper as the Sudan method, sedimentation is employed for the extraction of clay and silt. The pipette technique is not favoured because there may be soils which like the Sudan soils may refuse to disperse completely at one operation, whereas the repeated treatments involved in the sedimentation process further disperse soil particles not fully dispersed in the first operation. But the principal objection to analysis by sedimentation is that it takes considerable time to be completed, so that unless it is found absolutely necessary the rapid pipette technique should be adopted wherever possible.

It has been stated above that the acid treatment in the International method is included mainly for the purpose of destroying such cementing materials as calcium carbonate, etc. Laterites contain little or no calcium carbonate and little exchangeable bases (Table I). The acid treatment therefore appears to be superfluous for such soils. Accordingly it was proposed to test the dispersion by shaking the soil with NaOH directly. In this method which will be termed direct NaOH method, 20 grms. of soil were taken in a two-litre narrow mouth bottle to which were added 4 c. c. of normal NaOH and 500 c. c. water. The mixture was then shaken in an end-over-end-shaker for 24 hours revolving at about 40 revolutions per minute. It was then diluted to two litres and the usual pipette technique followed for the estimation of clay and silt.

In all, therefore, the following four methods were tried at first which briefly stated were as follows :—

(1) International method (HCl-NaOH)—pipette technique.
(2) Puri method (NaCl-NaOH)— ,, ,,
(3) Direct NaOH method (NaOH)— ,, ,,
(4) Sudan method (Na_2CO_3)—Sedimentation procedure.

There are of course a number of other methods of mechanical analysis, but it was expected that the above four methods would suffice to bring out the behaviour of lateritic soils and enable further modification in those methods to be made if necessary.

Soil samples.

At our request the Provincial Agricultural Departments sent us samples of lateritic soils which they considered typical of their respective provinces. From

amongst these, 18 samples were chosen to represent the whole lot and the results of their analysis are reported in this paper. These samples were all passed through 2 mm. sieve. In Table I below are given the locality from which these samples were collected, their colour, loss on ignition, organic matter determined by the wet oxidation method [A. O. A. C. 1925; Read and Reddgell, 1922], carbonate content by Collin's calcimeter, total exchangeable bases by electro-filtration, pH values by H-electrode, etc., from which it will be found that Indian laterites generally contain little organic matter, and are extremely poor in lime content and exchangeable bases.

TABLE I.

Showing organic matter, lime content, exchangeable bases, pH, etc., in Indian laterites.

Soil No.	Lab. No.	Soil from	Colour	Organic matter per cent.	Loss on ignition per cent.	Moisture in air-dry soil per cent.	Carbonate as $CaCO_3$ per cent.	Total exchangeable bases in milli-equiv. per 100 grms. air-dry soil	pH
1	34	Calicut, Madras	Red	1·9	16·5	4·0	trace	1·4	5·1
2	58	Kumata, Bombay.	Red	2·4	14·8	5·6	*nil*	5·7	6·0
3	64	Tenasserim, Burma	Dark brown	4·1	10·8	3·0	*nil*	2·0	5·7
4	74	Bhubaneswar, Orissa.	Light yellow with a tinge of brown	0·9	5·8	2·6	*nil*	4·5	5·5
5	24	Raipur, C. P.	Red	1·1	9·7	1·9	0·034	3·9	6·6
6	30	Teliparamba, Madras.	Dark brown	10·2	26·9	6·3	trace	2·7	5·4
7	80	Satgaon, Assam	Light red	1·8	11·9	4·2	trace	0·6	5·0
8	1	Giridih, Bihar	Deep red	*	5·6	2·9	trace	4·6	7·2
9	5	Giridih, Bihar	Red	..	5·9	3·3	*nil*	1·7	6·7
10	11	Deoghar, Bihar	Yellowish brown	..	5·5	2·0	*nil*	3·3	6·7
11	15	Deoghar, Bihar	Red	0·4	7·4	2·9	trace	2·5	6·8
12	98	North Haji Dacca Farm.	Light yellow with tinge of brown	1·3	5·2	1·8	*nil*	1·7	5·6
13	100	Khoskhana Dacca Farm.	Ditto	1·9	7·8	2·8	*nil*	1·7	5·0
14	26	Guntur, Madras	Deep red	..	3·8	1·4	trace	4·2	7·7
15	20	Insein, Burma	Light red	..	4·0	1·0	trace	1·3	5·3
16	38	Bankura, Bengal	Do.	0·3	4·7	1·5	*nil*	3·1	6·4
17	44	Birbhum, Bengal	Light yellow with tinge of brown	..	2·8	1·1	*nil*	2·7	6·1
18	53	Midnapore, Bengal	Ditto	..	2·8	1·0	*nil*	2·6	6·3

* Organic matter was not determined in this and other soils where the percentage loss on ignition was small.

EXPERIMENTAL RESULTS AND DISCUSSION.

In Table II below are given the percentages of clay and clay + silt, obtained by the four methods of mechanical analysis as stated above, in the 18 soils under investigation.

TABLE II.

Percentage clay and clay + silt in air-dry soil.

Soil No.	Clay per cent.				Clay + silt per cent.			
	International method (HCl-NaOH)	Puri method (NaCl-NaOH)	Direct NaOH method	Sudan method (Na_2CO_3)	International method (HCl-NaOH)	Puri method (NaCl-NaCH)	Direct NaOH method	Sudan method (Na_2CO_3)
1	43·8	9·0	43·5	44·2	57·5	17·5	57·6	56·9
2	43·6	16·3	43·4	43·8	65·1	31·4	66·3	64·5
3	19·5	5·7	19·2	19·9	27·3	16·2	28·8	27·7
4	17·2	12·6	16·3	17·1	36·4	35·8	38·6	36·4
5	17·1	10·4	16·6	14·9	31·3	23·7	32·7	...
6	41·9	6·4	41·2	42·6	66·9	14·8	64·8	63·5
7	27·3	15·0	27·9	...	47·9	41·0	48·6	...
8	21·2	21·8	22·1	20·5	29·4	31·3	31·8	29·7
9	30·3	29·9	31·7	31·3	42·8	43·5	43·7	43·5
10	21·1	21·3	21·4	22·3	36·4	38·2	35·5	36·7
11	29·2	31·1	31·1	30·3	41·8	44·3	44·2	42·0
12	18·4	18·8	17·7	17·2	51·1	52·9	53·6	...
13	25·3	25·4	25·6	24·2	56·7	58·8	59·9	...
14	12·0	12·8	11·1	...	16·0	16·0	15·0	..
15	12·6	10·7	10·8	...	18·9	19·2	19·6	...
16	19·0	17·7	17·7	...	30·0	27·0	29·2	...
17	9·2	10·1	9·6	...	23·7	24·9	25·5	...
18	10·5	10·3	10·0	...	22·1	23·7	23·2	...

It is evident from the above table that the International, direct NaOH and the Sudan methods gave almost equal results. Consequently for lateritic soils, which

contain little or no calcium carbonate and very little exchangeable bases (Table I), there is no obvious gain by introducing the HCl treatment as in the International method, or by following the sedimentation process as in the Sudan method. On the other hand, the fact that almost equal amounts of clay were obtained by different methods, shows that the dispersion in every case was almost complete or at any rate reproducible.

Puri method, however, failed to disperse the first seven soils adequately although it gave results as good as those obtained by other methods for the remaining soils. The dispersion in the International and the direct NaOH method was effected by 24 hours' shaking of the soil suspension, made alkaline by 4 c. c. of normal NaOH solution, whereas in the Puri method the suspension was made just alkaline to phenolphthalein (about 1 c. c. of *N* NaOH was required) and then left for 5 to 6 hours with occasional hand-shaking. In order to see, therefore, whether dispersion by Puri method, improved by employing the mechanical shaking and also by using a larger quantity of alkali, four soils 1, 2, 3 and 6 selected and dispersion treatments as stated in Table III below were tried.

TABLE III.

Percentage clay and clay + silt obtained by employing different dispersion treatment.

Soil No.	International method using 4 c.c. *N* NaOH	Direct NaOH method using 4 c.c. *N* NaOH	Puri method, suspension just alkaline	Puri method suspension shaken 24 hrs. after making just alkaline	Direct shaking of suspension for 24 hrs. after making just alkaline	Puri method suspension shaken for 24 hrs. with 4 c.c. *N* NaOH
			Clay per cent.			
1	43·8	43·5	9·0	22·7	26·1	43·4
2	43·6	43·4	16·3	31·8	25·7	44·2
3	19·5	19·2	5·7	12·2	11·3	19·1
6	41·9	41·2	6·4	26·3	28·0	38·2
			Clay + silt per cent.			
1	57·5	57·6	17·5	54·4	50·4	58·5
2	65·1	66·3	31·4	64·9	60·7	67·0
3	27·3	28·8	16·2	25·5	24·6	28·2
6	66·9	64·8	14·8	55·4	52·8	61·1

Comparison of results given in column 5, in Table III above, shows that better dispersion of soil is obtained by adding the standard shaking procedure extending over 24 hours to the Puri method. Even then the amount of clay so obtained is much smaller than that given by the International or the direct NaOH method. As a matter of fact, it is almost equal to that given by shaking

the soil suspension directly with that amount of alkali as has been used in the Puri method without the NaCl treatment (column 6). But when 4 c.c. *N* NaOH and 24 hours' shaking are employed in the Puri method, the amount of clay thus obtained is practically equal to that given by the International or the direct NaOH method. It is clear, therefore, that the incomplete dispersion of some of the lateritic soils obtained by following the Puri method as shown in Table II is obviously due to insufficient quantity of NaOH used and the lack of mechanical shaking. When these are rectified the Puri method is as good as the International method.

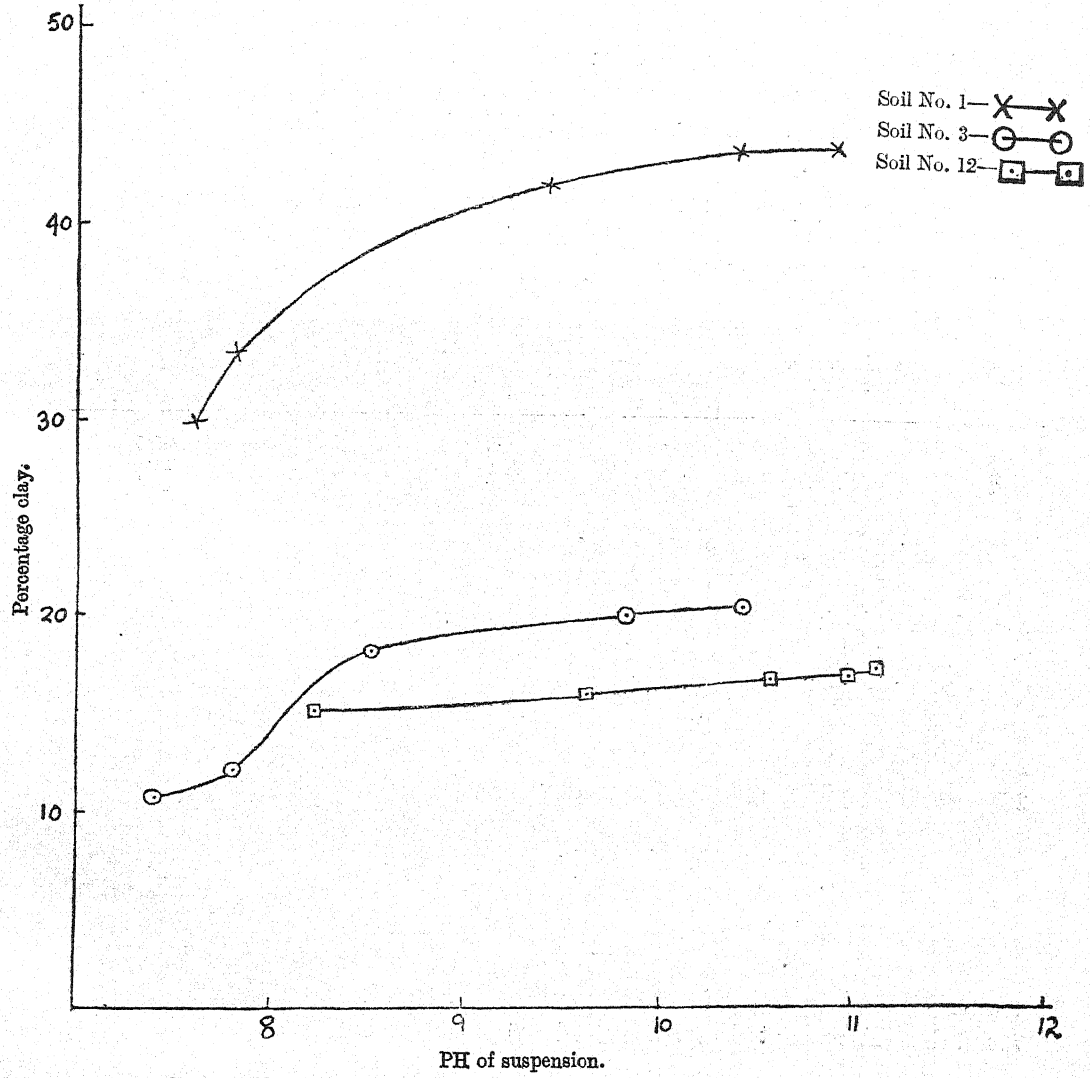


Fig. 1.—Showing the dispersion of soil in aqueous suspensions of varying pH.

It appears from above that dispersion is considerably affected by the quantity of NaOH used. Attempt was therefore made to find out the amount of alkali which would effect complete dispersion without being too drastic for lateritic soils. For this purpose suspension of 20 grms. lots of soils 1, 3, and 12 were shaken with 1, 2, 4, 6 and 8 c.c. of *N* NaOH solution for 24 hours and the clay determined. The pH of the suspensions was measured with hydrogen electrode. In Fig. 1 the clay figures are plotted against the pH of the suspensions from which it will be found that the former practically reached the maximum value at pH 10-10·5. We have also found that the amount of soil material other than organic matter in solutions having pH upto 10·5 does not exceed 0·6 per cent. Consequently we consider that the quantity of NaOH to be used for dispersion should be such that the resulting reaction of the suspension does not differ much from pH 10·5. Some 6 to 8 c.c. of *N* NaOH to 20 gms. soil will be found suitable for this purpose with Indian laterites.

USE OF H_2O_2 IN THE MECHANICAL ANALYSIS OF LATERITIC SOILS.

TABLE IV.

Percentage of clay obtained by the same quantity of alkali before and after H_2O_2 treatment.

Soil No.	Organic matter per cent.	International method HCl—NaOH 8 c.c. *N* NaOH	Direct NaOH method 8 c.c. *N* NaOH	International method H_2O_2—HCl—NaOH 8 c.c. *N* NaOH	Direct NaOH method H_2O_2—NaOH 8 c.c. *N* NaOH
2	2·4	45·5	44·9	46·2	45·5
3	4·1	21·9	21·5	18·4	18·9
6	10·2	45·7	43·5	48·1	46·9

It will be seen from the above table that after the H_2O_2 treatment higher clay figures for the soil Nos. 2 and 6 and lower clay figure for the soil No. 3 were obtained by both the International and the direct NaOH method. It should be mentioned that, as a result of the H_2O_2 treatment, the percentage of clay for all the above soils was reduced by amounts equal to : (1) percentage loss of clay by solution due to H_2O_2—0·2 per cent., 1·6 per cent. and 3·1 per cent. for soils 2, 3, and 6 respectively—and (2) percentage loss of organic matter—0·7 per cent., 2·5 per cent., and 4·7 per cent. for soils 2, 3 and 6 respectively—which otherwise would have remained in solution due to the alkali and been added, although improperly, to the clay figure. Obviously, therefore, in the case of soil Nos. 2 and 6, improvement in dispersion due to the use of H_2O_2 has more than counterbalanced the losses mentioned above, while in the case of soil No. 3 this has not been so.

Although it appears from the above, that a considerable amount of organic matter comes in solution in the direct NaOH method, nevertheless it has been found that this amount does not exceed 0·5 per cent. in case of soils containing

organic matter 2 per cent. or less. Consequently the direct NaOH method may be employed for mechanical analysis of Indian lateritic soils which generally contain less than 2 per cent. organic matter (Table I). For soils with higher percentages of organic matter hydrogen peroxide treatment should be included in the method.

Short description of the direct NaOH method.

Twenty grms. of lateritic soil are shaken with 500 c.c. of water and 6 or 8 c.c. of *N* NaOH solution (so that the suspension has a pH about 10·5) for 24 hours in an end-over-end shaker. The suspension is then passed through a 0·2 m. m. sieve (standard 70 mesh I. M. M.) and made up to 2 litres. Clay and silt are estimated by employing pipette technique, and fine sand by sedimentation [1928]. For soils containing more than 2 per cent. organic matter, hydrogen peroxide treatment should precede the shaking with alkali. In this case, however, the loss by solution of sesquioxide, etc., should be determined, by igniting the precipitate obtained by addition of ammonia in the filtrate after the peroxide treatment, and added to the clay figure.*

General remarks.

The results of the present investigation show that contrary to the statements mentioned elsewhere [Keen, 1931; Rep. A. E. A., 1928; Robinson, 1931], lateritic soils offer little difficulty for mechanical analysis. It is believed that these statements were made from *a priori* reasoning since no data of mechanical analysis were available at that time for this type of soil which was known to possess a highly granulated structure. The few different methods of analysis that were tried here on Indian laterites gave the same amount of clay, that is to say, the dispersion in every case was complete or at any rate reproducible. Amongst these methods the direct NaOH method, a short description of which is given above, has been found to be the most simple and the least time-consuming, and is therefore likely to prove very suitable for the mechanical analysis of Indian laterites where complete dispersion is aimed at.

Summary.

1. The following methods of mechanical analysis were tried on 18 samples of lateritic soils from all parts of India—

(i) International method without the peroxide treatment—HCl-NaOH.

(ii) Puri method—NaCl-NaOH.

(iii) Sudan method—Na_2CO_3.

Since these lateritic soils were found to contain very little calcium carbonate and exchangeable bases

* Should the clay complex contain a large amount of exchangeable calcium, the HCl treatment should be included in order to avoid the flocculating effect of the calcium hydroxide produced by the addition of alkali. So far as the Indian laterites are concerned, the HCl treatment has been found unnecessary.

(iv) a direct NaOH method was tried in which the soil was shaken directly with NaOH without the previous HCl or NaCl treatment as in (i) or (ii) above.

2. The International, Sudan and direct NaOH methods gave almost equal amounts of clay and silt in all cases. Puri method, however, gave a lower clay figure for some of the soils. This was due to the insufficient quantity of alkali used for dispersion, and lack of mechanical shaking in the method as prescribed by the author.

3. Maximum dispersion of lateritic soils was found to take place in suspensions having pH about 10·5. Consequently the quantity of NaOH to be used for dispersion should depend on this factor rather than on any arbitrary amount. In most cases 6 to 8 c.c. of *N* NaOH to 20 grms. of soil was found suitable for the purpose without being drastic.

4. For lateritic soils containing more than 2 per cent. of organic matter it is desirable to include the peroxide treatment.

5. The direct NaOH method is found to be reliable and less laborious for the mechanical analysis of lateritic soils. A brief description of the method is given.

ACKNOWLEDGMENT.

This investigation was carried out at the Agricultural Research Section of the University of Dacca and authors take this opportunity to express their indebtedness to Mr. G. H. Langely, the Vice-Chancellor and Professor J. C. Ghosh, Head of the Department of Chemistry, for taking continued interest in the progress of the work and to the Imperial Council of Agricultural Research for financial help towards the purchase of necessary apparatus and the maintenance of the salary of one of us (J. N. C.).

REFERENCES.

Charlton, J. (1927). *I. I. Agric. Res., Pusa Bull.* No. 172.

Joseph, A. F. and Snow, I. W. (1929). *Jour. Agric. Sci.* **19**, 106.

Keen, B. A. (1931). "The Physical Properties of the soil". Longmans, Green & Co., London, p. 78.

Mattson, S. (1927). *Proc. First. Intern. Soc. Soil Sci.*, II, 185.

Methods of Analysis (1925). A. O. A. C. Washington D. C., Second edition, p. 25.

Olmstead, L. B., Alexander, L. T. and Middleton, H. F. (1930). *U. S. Dept. Agric. Tech. Bull.* No. 170.

Puri, A. N. and Bhailal, M. A. (1928). *I. I. Agric. Res., Pusa Bull.* No. 175.

Puri, A. N. (1929). *Mem. Dept. Agric. Ind., Chem. Ser.*, **10**, 209.

Read, J. W. and Riddgell, R. H. (1929). *Soil Sci.*, **13**, 1.

Report of the sub-committee of Agricultural Education Association (England) (1926). *Jour. Agric. Sci.* **16**, 123.

Report of the sub-committee of A. E. A. (England) (1928). Agricultural Progress **5**, 137.

Robinson, G. W. (1921). *Imp. Bur. Soil Sci.* (England). *Tech. Com.*, No. 17, p. 8.

DETERMINATION OF NITROGEN IN SOILS. I.

BY

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Although a very large part of modern agricultural research relates to the study of nitrogen transformations in soils, yet the conditions relating to the estimation of total nitrogen have not, so far, been standardised. The accuracy of the estimate of nitrogen present in a plot of land will be determined by (*a*) the method of collecting specimens of the soil, (*b*) the number of specimens per unit area, (*c*) the technique of sampling, (*d*) the size and number of specimens analysed, (*e*) the nature and composition of the soil, and (*f*) the method of analysis. The errors inherent to the methods of collection of specimens and sampling for analysis are too well-known to require repetition. Those relating to the size and number of specimens have also been recognised, particularly since the introduction of statistical methods in recent years. The effects of the nature and composition of the soil on the accuracy of the estimate of nitrogen have not, however, been, so far, fully understood. Nor are the methods of analysis sufficiently improved to warrant accurate estimates of the nitrogen contents of the specimens analysed. The Kjeldahl method, with its various modifications, is the one generally adopted for analysis, and although it is reliable for estimating nitrogen contents of easily decomposable organic substances, it is not, however, sufficiently accurate for detecting small changes in the nitrogen content of the soil.

Working with black cotton soils, Bal [1925] observed that the Kjeldahl method gave consistently low values for nitrogen when the soils were digested in the usual way, but that higher and more consistent figures could be obtained if the soils were moistened with water before digestion. He also noted that by treating the undigested residue with water, then retreating with concentrated acid and distilling the ammonia in the usual way, a iurther amount of nitrogen was recovered. This increase added to the amount originally found by the "dry" method, was practically equal to the amount of nitrogen found in the soil when digested originally by the "wet" method. He attributed the incompleteness of the digestion in the case of "dry" method to the existence of some cementing material, which does not dissolve in the concentrated acid, but does so in the dilute one.

In the course of his investigations on nitrogen transformations in swamp soils, the author encountered difficulties in obtaining accurate estimates of the total nitrogen contents of his specimens. An investigation was therefore undertaken with a view to study the exact conditions under which a complete digestion is ensured, and the present paper gives a preliminary account of certain observations indicating (*a*) some modifications that would help to improve the efficiency of digestion and yield more reliable and consistent values than would otherwise be possible, and (*b*) the probable nature of the factors influencing the accuracy of the estimate of total nitrogen in a specimen of soil.

Experimental.

A specimen of fairly heavy clay soil from Tellicherry, in South India, was air-dried, ground and passed through a 30-mesh sieve. Samples (5 grms.) of the above were then digested and their nitrogen contents determined, by the Gunning and Hibbard modification of the Kjeldahl method [1925]. Back titrations of unused acid were carried out against N/30 alkali.

With a view to test the efficacy of the addition of water and the effect of time of standing with water, varying amounts of ammonia-free water, followed by 20 c. c. of concentrated sulphuric acid in each case were added to similar quantities of soil, and the digestions conducted either immediately or after being allowed to stand overnight. In the latter case, the flasks were stoppered to avoid absorption of ammonia from the air. The results are presented in Table I. The average figures for replicate determinations are given along with the standard deviation in each case.

Table I.

Effect of moistening with varying amounts of water on the estimate of nitrogen present in a soil.

Vol. of water added in c.c.	0	5		10		15		20	
		Nitrogen as parts per million							
	Untreated control	Imm.*	O. N.†	Imm.	O. N.	Imm.	O. N.	Imm.	O. N.
Average value	966·8	980·4	1028·5	1005·0	1028·3	1023·3	1033·0	1049·0	1044·0
Standard deviation	±18·1	±39·7	±7·8	±30·1	±9·2	±26·6	±14·1	±8·7	±8·7

* Imm.—Digested immediately after moistening.
† O. N.— „ after standing overnight.

It would be seen from the above that not only higher, but also more consistent figures have been obtained by treating with increasing amounts of water up to 20 c. c., and that allowing the mixture to stand overnight before digestion generally shows improvement in the values: in the last case, however, there was some slight but not appreciable difference between the effects of immediate digestion and that after standing overnight. It was also noted that, with increasing addition of water up to 20 c. c., the digestion proceeded more smoothly and at a faster rate than in the untreated ones. That the digestion was more complete as the quantity of added water increased, was also manifest from the appearance of the residue left in the digestion-flask after repeated washing and decantation previous to distillation with alkali, for while in the untreated cases the residue was coarse, gritty and somewhat dark-coloured, it was fine, sandy and almost perfectly white in the treated ones.

Increasing the volume of added water beyond 20 c. c. did not lead to any further increase in the corresponding values for nitrogen. Thus, with 30 c. c., the following values were obtained:—immediate, 1028·0; overnight, 1039·0. In view of the above and the fact that increasing volume of water would necessitate longer heating to evaporate it prior to addition of salt mixture, the observations were not extended in that direction.

Bal's observations having suggested that the cementing materials are soluble in dilute acid, some experiments were carried out to determine whether previous treatment of the soil with dilute sulphuric acid of varying strengths would result in any further improvement. In all cases the digestions were carried out either immediately, or after standing overnight, the necessary further amount of concentrated acid being added during the course of digestion after the fumes began to appear. The mean values are set forth in Table II.

TABLE II.

Effect of preliminary treatment with dilute acid.

Acid per cent. by weight	Nitrogen as parts per million	
	Immediate	Overnight
18·5	995·5	994·0
15·0	1034·0	1023·0
12·0	1034·0	1034·0

It will be observed from the above figures that the results are not so high as those obtained by some of the treatments described in the previous experiments. They suggest that the cementing materials, if any, are not, readily soluble in dilute acids of the strengths tried in the present experiment.

From the foregoing observations it would appear that the best method of obtaining an accurate estimate of the nitrogen content is to treat the soil (5 grms.) with 40 c. c., of 1 : 1 sulphuric acid and conduct the digestion either immediately or after allowing the mixture to stand overnight. Whenever possible the latter procedure is to be preferred on account of the greater ease of digestion in that case.

With a view to determine whether these conditions hold similarly with other soils as well, trials were carried out with four different specimens of soils. Table III gives a summary of the results obtained together with the standard error of the mean in each case.

TABLE III.

Nitrogen in different soils as compared by "wet" and "dry" methods. Average values expressed as parts per million.

Method	Nasik dryland—surface soil	Dacca highland—surface soil	Punjab riceland—surface soil	Mandalay riceland—surface soil	Standard error of the mean—p. p. m.
Official, Gunning—Hibbard	858·5	819·5	944·0	472·0	±2·7
Do. modified—moistened with 20 c. c. water—Imm. . .	880·5	842·5	985·0	543·5	±1·1
Do. 20 c. c. water—O. N. .	908·0	839·5	988·0	541·0	±0·9

It may be noted that the dry method gives consistently lower figures for all the soils experimented with. The difference between the effects of immediate "wet" digestion and that after standing overnight are not significant in some cases, but a critical study of the results would, however, show that even in such cases the latter treatment gives slightly more concordant results than the former. Moreover, as no definite information is yet available regarding the relation between the nature of the soil and the efficiency of its digestion by any one of the methods, it would be desirable to conduct all digestions after standing overnight.

In view of the observations that all the specimens of soils experimented with, gave lower values by the official "dry" method, than by the "wet", it appeared probable that certain substances common to all the soils may actually be responsible for the hindering of the proper digestion by the former method. Since the

oxides of silicon, iron and aluminium are present in all cases, it was considered probable that variations in the quantities of these substances may influence the accuracy of the estimate of nitrogen. Some experiments were accordingly carried out with the Tellicherry soil used in the first experiment, to determine the effect of addition of the above-mentioned oxides (1 grm. in each case) on the results obtained by the two methods. The results are shown in Table IV.

TABLE IV.

Effect of addition of different oxides on nitrogen values.

Treatment	Nitrogen as parts per million				
	Soil alone (control)	Soil + Fe_2O_3	Soil + Al_2O_3	Soil + SiO_2	Standard error of the mean p. p. m.
"Dry" digestion . .	976·9	949·1	954·8	987·8	±2·05
"Wet" overnight . .	1046·5	1039·0	1055·0	1041·0	±2·84

It may be seen that addition of oxides of either iron or aluminium causes a marked fall in the estimate of nitrogen by the "dry" method. Addition of silica does not seem to make any appreciable difference. It is probably owing to the fact that the differences in the nitrogen values as obtained by wet and dry digestions are least manifest in the case of sandy soils [Bal, *loc. cit.*].

The results obtained by the "wet" digestion on the other hand, are not appreciably affected by the addition of the oxides. It is very clear that iron and aluminium oxides do, in some way, obstruct the progress of the digestion by the "dry" method. Since all soils contain these two oxides, though to varying extents, it would follow that the usual dry method is not adequate for obtaining accurate estimates of nitrogen contents of soils. The "wet" digestion is definitely an improvement on the older one and, in addition to giving higher values, also helps the digestion to proceed very much more smoothly and in less than half the time taken by the former.

The foregoing observations are essentially preliminary and only indicate the nature of the difficulties to be encountered, while suggesting certain modifications that would help to yield higher and more consistent values. Further work is in progress to (*a*) determine the precise nature of the compounds that interfere with the progress of digestion, (*b*) investigate the effect of addition of other mineral oxides and non-nitrogenous organic substances on the accuracy of the estimate of

nitrogen, and (*c*) standardise the conditions of digestion so as to suit all types of soils.

Summary.

1. Estimates of total nitrogen obtained by the usual dry digestion of soils with sulphuric acid are invariably lower than those obtained by digestion after wetting.

2. Among the treatments so far tried the best would appear to be that of moistening at the rate of 20 c.c. of water for every 5 grms. of soil, then adding concentrated acid (20 c.c.) and allowing the mixture to stand overnight prior to digestion.

3. Evidence has been obtained to suggest that the oxides of iron and aluminium which are present in all soils, are among the compounds that interfere with the progress of digestion by the usual method, but further work is needed to elucidate the nature of all the various non-nitrogenous compounds that are concerned in the obstruction and to determine the mechanism of such action.

4. Attempts are being made to throw light on the above and to standardise the conditions of estimation so as to ensure the true values being obtained for all types of soils.

The author's thanks are due to Dr. V. Subrahmanyan for his interest in the progress of the work.

References.

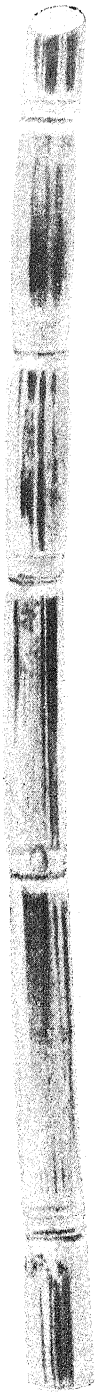
Bal, D. V. (1925). *J. Agric. Sci.* **15**, 454.

Gunning, J. W., and Hibbard, P. L. (1925). Methods of Analysis, A.O. A.C., Washington.

PLATE LIV. Co. 213 and its bud variations.



Co. 213.



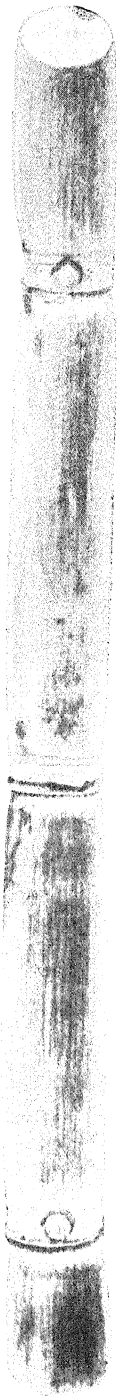
Striped Sport.



Thin Yellow Sport.



Thin Purple Sport.



Thick Purple Sport.

BUD VARIATIONS IN CO. 213 SUGARCANE.

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(Received for publication on the 4th July 1932)

(With Plates LIV—LVI).

I. INTRODUCTION.

The occurrence of *vegetative*, *clonal* or *bud* sports in the sugarcane has been known for a long time. One of the first definitely recorded instances is that by M. Louzier in Mauritius about the year 1869 which resulted in the establishment of a variety of that name in that island. Other well known instances of sporting in the cane are those of the "Ribbon" canes in Australia, the "Tanna" canes in Mauritius and the "Tip" canes in Hawaii. In such sporting a complete cycle is often obtained, a striped cane throwing out uni-coloured bud sports and these, in their turn, occasionally throwing out the original striped cane. In recent years sporting in certain P.O.J. canes has been recorded by Cross [1928] and Schultze [1930].

In India the earliest to definitely record and describe sporting in sugarcane was Barber [1906] who isolated from more than one striped cane at Samalkota a certain number of sports on the basis of colour. These were found to differ in other agricultural characters as well; one of these under the name '*Gillman*'—a uni-coloured red sport from Striped Mauritius—figured in certain of the agricultural stations in India for a time. Similar sports have been reported from other agricultural stations also from time to time; but none of these has definitely taken hold on cultivation as varieties superior to the extant kinds.

With the discovery of the fertility of seeds in the sugarcane and the breeding of valuable seedling canes that followed from it, the exploitation of bud sporting as a means of evolving improved strains has, naturally, taken a secondary place. Recently, however, interest in this line of work has somewhat revived. It has been rightly argued that, though colour sporting is the one most easily noticed, such a phenomenon very likely connotes the possibility of sporting in such crop characters as juice quality, habit, growth vigour and disease resistance. Of work of this nature in recent years mention needs to be made of the Hawaiian work by Shamel, Verret and Paris [1923] and by Moir [1932] and of work in Mauritius by Hill [1930].

The present paper embodies observations on a rather complete cycle of bud sporting in the Coimbatore cane, Co. 213, extending over a period of five years. My attention to sporting in this cane was first drawn by Noel Deerr in 1924. That year he sent for experimental growing at Coimbatore material which included canes resembling the *purple* sports described in this paper.

II. History of the sports.

During the season 1926-27 a plot of Co. 213 happened to get planted on land, not the most suitable for cane growing. Growth in this plot was consequently below the average for this cane at Coimbatore. One of the clumps, out of a total of sixty thus planted, showed on examination one rather poorly developed cane which was distinctly, though rather lightly, striped—reddish or brownish purple stripes on a background of straw yellow. The other canes in the clump and in the row were coloured like Co. 213 at Coimbatore, *viz.*, uni-coloured reddish or brownish purple. After ruling out possibility of mixture by a careful dissection of the clump, this cane was cut into one-budded setts and planted in good sugarcane soil in April 1928, yielding a row of six plants. During the crop season 1928-29, it was noticed that the row of plants of this new sport looked different from the plot of Co. 213 planted alongside of it; the growth was poorer and the leaves shorter and narrower with more erect leaf tips. This appearance intensified interest in the sport and led to a careful examination of all the six clumps. This examination was rewarded by the discovery of one clump which showed the undermentioned canes :—

(*a*) Nine striped canes similar to the ones originally planted. This will hereafter be called in this paper "*Striped Sport*".

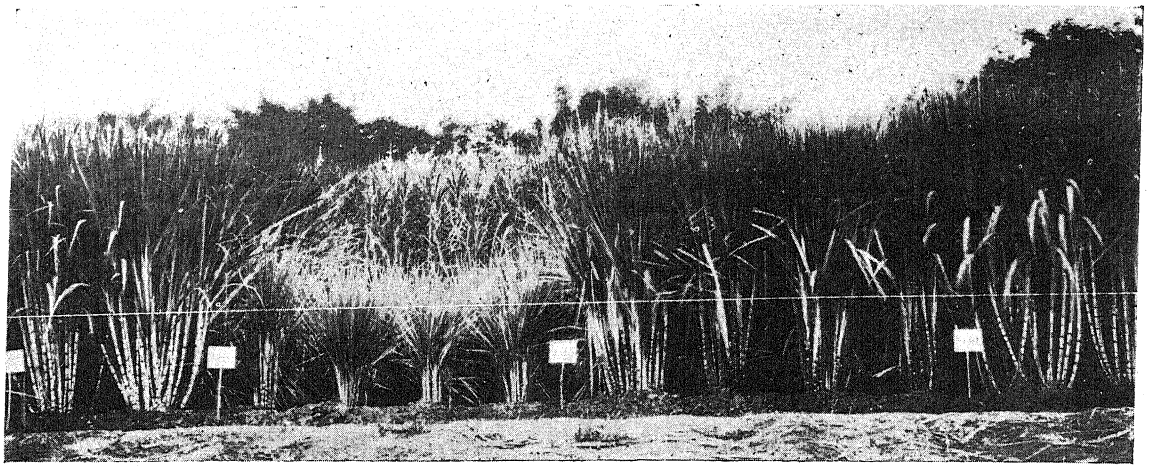
(*b*) One cane which in colour and thickness was very similar to the original Co. 213, to be called hereafter "*Thick Purple Sport*".

(*c*) Two canes coloured like Co. 213 but with distinctly thinner canes and shorter narrower leaves, to be called hereafter "*Thin Purple Sport*".

(*d*) Two canes very similar to the *Thin Purple Sport* but wax-yellow in colour, to be called hereafter "*Thin Yellow Sport*".

Four different bud variations had thus been obtained from Co. 213.

All the four sports, with the original Co. 213 for comparison, were grown for a couple of seasons—1929-30 and 1930-31—in adjoining rows; and it was found that all the sports grew true to type except for the fact that, whereas the *Thin Yellow Sport* lost steadily in vigour at each planting, the others—particularly the *Thin Purple Sport*—gained in this respect. It was unfortunate that weights were not recorded at the time; the scientific interest of the sports was not fully realised then. During the 1931-32 season, however, all the four sports were grown in fair



Thin Purple Sport. Thin Yellow Sport. Striped Sport. Co. 213.

Fig. 1.

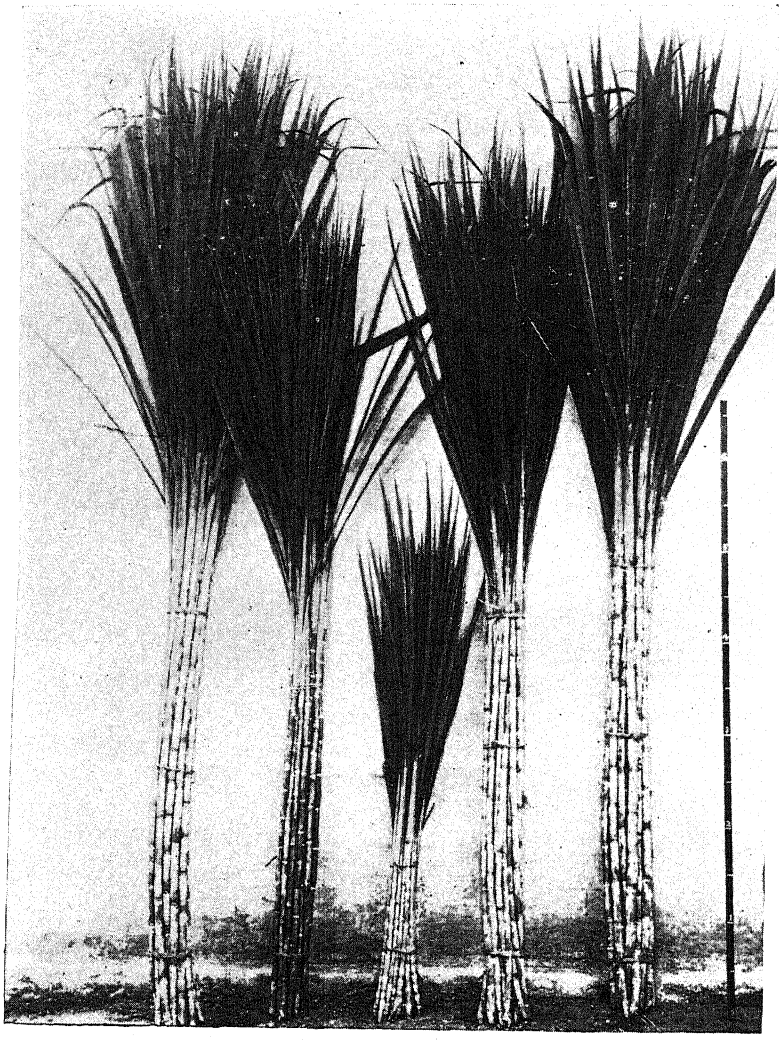


Fig. 2.—The cane bundles from left to right are of Co. 213, Striped Sport, Thin Yellow Sport, Thin Purple Sport and Thick Purple Sport.

quantities—four twenty-plant rows of each and repeated three times—and careful notes recorded on their characters.

III. Description of the Sports.

(*a*) *Growth vigour.*—The first character to be noticed was the markedly poor vigour of the *Thin Yellow Sport* as compared with the rest (Pl. LV). The *Striped Sport* was a little inferior in this respect to the other two which were about equal to each other and to the original Co. 213. This difference was noticed in all the three plots and is evident from the weights recorded (Table I).

(*b*) *Lamina.*—The *Thin Yellow Sport* had the shortest and narrowest leaves The leaves of the *Striped Sport* were shorter and narrower than those of the other two which again were similar to the original Co. 213 (Table I).

The leaf tips were erect in the *Thin Yellow Sport* and the *Striped Sport*, the other two sports being nearer to Co. 213 in this respect (Pl. LV).

(*c*) *Juice quality.*—Analysed at the end of one year from planting, the *Thin Yellow Sport* was found to have the poorest juice (Table II).

(*d*) *Root—system.*—Root dissections were made in all the three plots and of all the four rows in each plot. The root system of the *Thin Yellow Sport* was again the poorest which explains the poor growth vigour of this sport.

Table I.

Weights and leaf measurements of the four "Sports" from Co. 213.

—	Average weight per row of above—ground portion	Average weight per row of millable canes	Leaf length	Leaf width
	lbs.	lbs.	ft. in.	in.
Co. 213.	152	91	4 7	2·1
Striped Sport	134	87	3 9	1·4
Thick Purple Sport . . .	154	95	4 6	2·1
Thin Purple Sport . . .	149	94	4 8	2·1
Thin Yellow Sport . . .	13	3	3 0	1·1

N.B.—The lengths and widths of leaves are the averages of 100 measurements.

TABLE II.

Juice analysis of the four "Sports" from Co. 213.

—	Brix	Sucrose	Glucose	Coefficient of purity
	per cent.	per cent.	per cent.	
Co. 213	19·74	17·48	0·36	88·5
Striped Sport	18·71	16·07	0·41	85·9
Thick Purple Sport	19·31	17·15	0·38	88·8
Thin Purple Sport	19·55	16·99	0·56	86·9
Thin Yellow Sport	15·70	12·09	1·18	77·0

IV. BUD VARIATIONS IN OTHER CANES.

Similar bud sports have been noticed in other canes as well and certain of these are mentioned below.

The cane, Co. 290, which is a uni-coloured 'glaucous green to greenish brown' cane has been found to throw out inferior striped canes, but the matter was not pursued further.

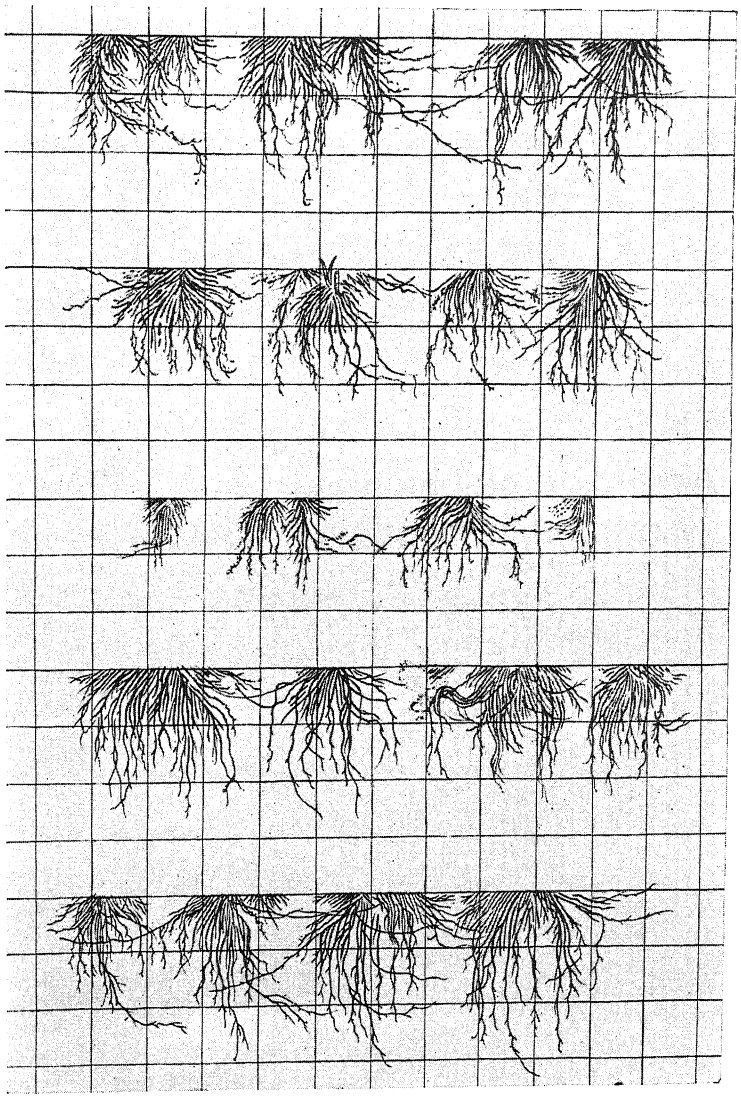
In the cane-fields round about Pilibhit in the United Provinces a variety called "*Reha*" is grown and is believed by the cultivators to be a distinct variety. In 1922 this was brought to Coimbatore. After about three years' growth at Coimbatore a bud sport was obtained which, on cultivation, proved very similar to *Saretha*, the variety so commonly grown in parts of the United Provinces. This might explain the manner in which *Reha* was originally obtained. From the crop point of view *Reha* is inferior to *Saretha*.

In 1924 a yellow sport was obtained from the striped cane called "*Patta Patti*" and grown extensively in Mysore. In 1911 a similar yellow sport was obtained from a striped cane called "*Namam*" grown in the Coimbatore district. Both the above sports proved inferior to the original canes and were dropped from cultivation.

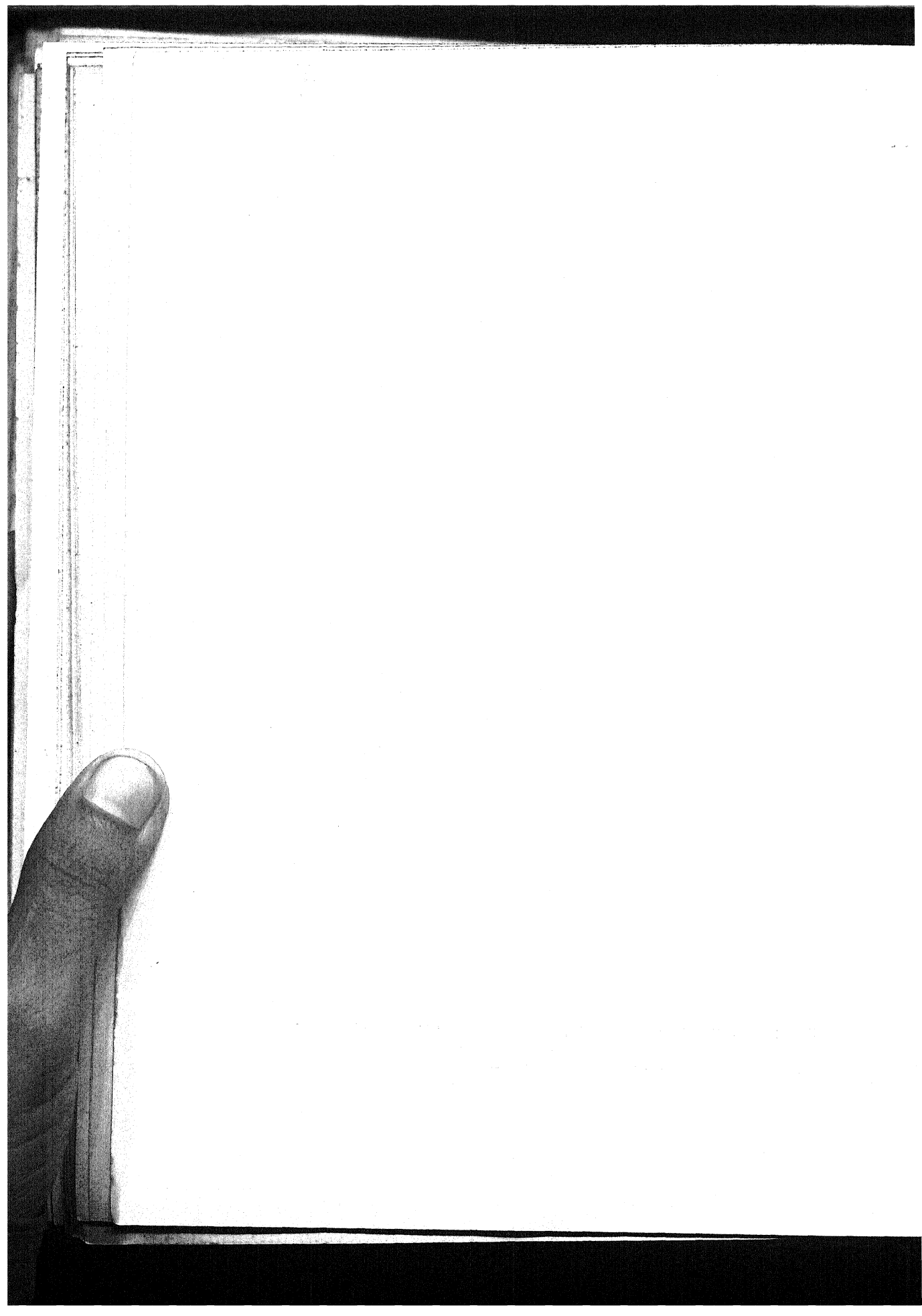
V. SUMMARY AND CONCLUSIONS.

A complete cycle of sports has been obtained from Co. 213 leading back to the original cane.

The *Striped Sport* and the *Thin Yellow Sport* are apparently degenerate bud variants, particularly the latter. This shows the importance of selecting proper material at the time of planting.



From top to bottom the roots are of Co. 213, Striped Sport, Thin Yellow Sport, Thin Purple Sport and Thick Purple Sport.
The background is in foot squares.



The *Thick Purple Sport* suggests the possibility of improving on this cane by bud selection.

The poor vigour of the non-purple or *Thin Yellow Sport* as compared with that of the two purple sports is in agreement with a similar experience in the P.O.J. canes 36 and 213 [Cross, 1928]

REFERENCES.

Barber, C. A. (1906). *Agric. J. Ind.*, 1, 285.
Cross, W. E. (1928). *Rev. Ind. Agric. Tucuman*, **18**, 141.
Hill, A. G. (1930). First Ann. Rep., Sugar. Res. Stat., Dept. Agric., Mauritius, for the year 1930, 9.
Moir, W. W. G. (1932). *Bull.* 8, *Fourth Cong., Intern. Soc. Sugar Tech.*
Schultze, E. F. (1930). *Rev. Ind. Agric. Tucuman*, 20, 149.
Shamel, A. D.; Verret, J. A. and Paris, F. A. (1923). *Intern. Sugar J.* 26, 412.

THE GREEN PEACH-APHIS (*MYZUS PERSICAE* SULZ.) AND A NEW PYRALID MANGO DEFOLIATOR (*ORTHAGA MANGIFERAE* N. SP.)

BY

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(Received for publication on the 2nd January 1932)

(With Plates LVII–LIX and one text-fig.)

The soil and the climatic conditions prevailing in the tract known as "Suresha" in Tirhoot in North Bihar are peculiarly favourable for fruit culture, and one who has lived long in the tract is struck with the ease with which fruit trees can be established in this area. The mango and the litchie grow to perfection in this tract. The peach, though not indigenous, when introduced at Pusa in the early decade of the present century was found to adapt itself so well that good fruits were produced which found a ready sale locally. Everything looked favourable for the extensive development of this particular fruit, when the fruit fly *Chœtodacus zonatus* Saund. appeared and did considerable damage to the fruit. The loss, by the fruit fly, was so great that, in subsequent years, the cultivation of the peach received a severe set back. When such was the condition, another serious pest, the Green Peach-aphis, *Myzus persicae* Sulzer, appeared suddenly in 1930 in large numbers. By the middle of March 1930, the majority of fruit trees were overrun by the aphis in every stage of its development. It was for the first time that the aphis was seen in such large numbers on peach trees in North Bihar. It reproduces itself parthenogenetically and in consequence large numbers of nymphs, wingless and alate females (Fig. 1), were found swarming on the trees, specially the tender, apical shoots. The young ones on hatching crawl about and establish themselves either on the cauline leaves or the tender fruits. Numbers of nymphs were to be seen congregated below flower buds. The fall of honey dew on the lower leaves was so profuse that they appeared shiny from a distance and attracted a host of ants and wasps. The nymphs were particularly seen to move upwards and preferred to establish themselves on the petioles and midribs of the tender apical leaves. Here they developed rapidly and in consequence innumerable

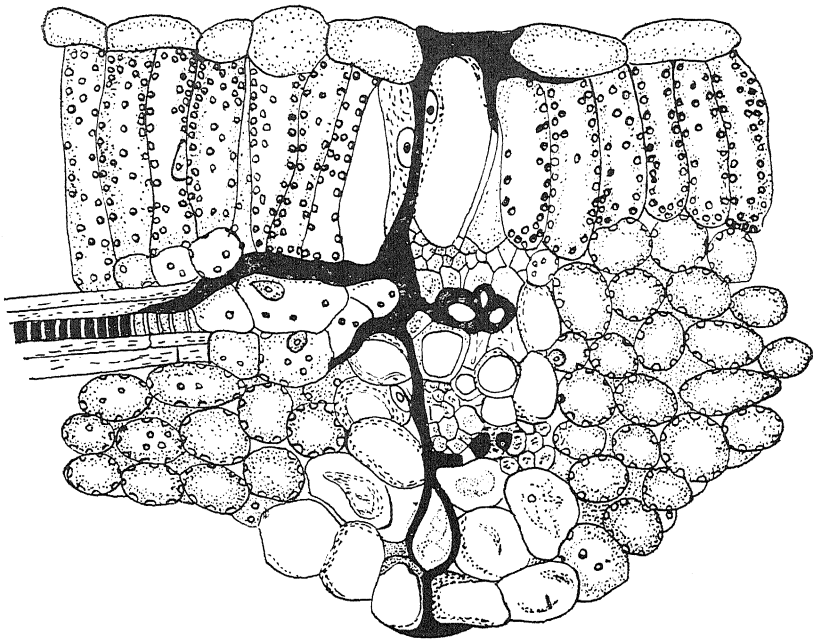


Fig. 1.

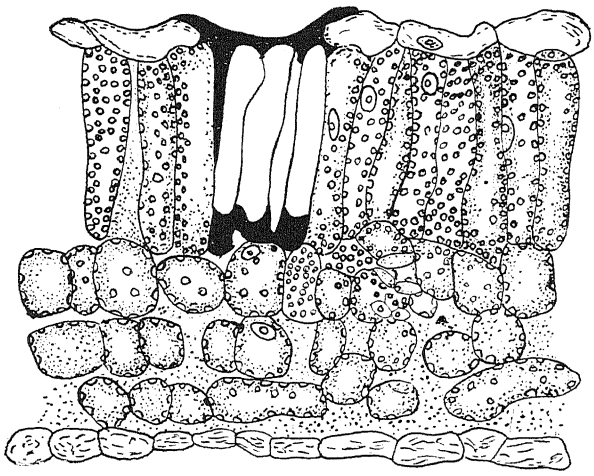


Fig. 2.

(For explanation please see page 540.)

exuvia were seen thickly scattered about on the leaves, which on account of the heavy drain of the sap became pitted and curled badly. The curling was more quick and lasting than any such phenomenon noticed in the past in the case of any other species of Aphidae infesting fruit trees or cultivated crops. The reason is that the nymphs drive their stylets deep through the epidermis and the cortex until they reach the phloem and in doing so inject saliva which is highly destructive to the surrounding cells which get plasmolysed and collapse early (Plate LVII, figs. 1, 2). The leaves become seared and drop off prematurely. The young setting fruits too cannot withstand the heavy drain. They shrivel up and drop down. In the majority of cases, where blossoming was in full swing before the advent of the Green Peach-aphis, there was hardly any setting of the fruit. Some of the heavily infested peach trees became so devitalized that they took a long time to recoup their lost vigour, and it was long after the rains before they put on their leafy appearance again.

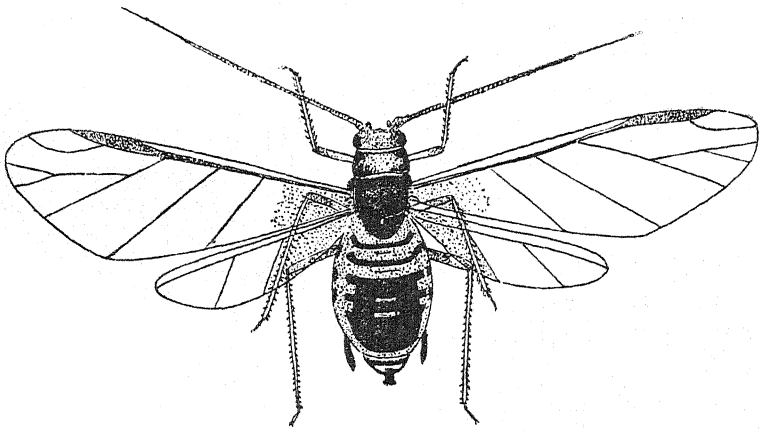


Figure 1.—*Myzus persicae* Sulzer.
Alate viviparous female

[After Theobald.

In this attack it was noticeable that, the aphis appeared suddenly, did the maximum amount of damage to fruits and trees, within the shortest time possible and disappeared, as suddenly as it had appeared, by the end of April, when the temperature rose suddenly high for a week or so. The infested trees drew myriads of Hover flies—*Syrphus balteatus* de Geer. and *Sphaerophoria nigritarsis* Brun. and lady-bird beetles, mostly *Coccinella sex-punctata* Linn. to them, but none of these predators was able to effect any appreciable improvement in the condition of the infested trees. By the end of April both winged (Fig. 1) and wingless viviparous females were seen to leave the infested trees in large numbers—so precipitately that

by the middle of May hardly any aphis was seen on the trees which a month before were teeming with millions of nymphs and adults. This aphis multiplies itself repidly at temperatures several degrees below that necessary to its insect enemies. A high temperature, with a corresponding low humidity has been observed to favour development. Sometimes two species occur on the same tree; the Green Peach-aphis and the Black Peach-aphis; the former is exclusively arboreal, whilst the latter, sometimes attacks the roots of peach trees which in consequence turn pale and do not fruit well.

The Green Peach-aphis is cosmopolitan in its distribution and polyphagous in its habits. It was first observed by Sulzer in 1761, who described it as *Aphis persicae* Sulz. [Sulzer, 1776]. It has been variously recorded in the eighteenth, nineteenth, and the present centuries as *Aphis dianthi* Schrank. It was also known as *Rhopalosiphum dianthi* Koch. and Van der Goot has described it recently as *Myzoides persicae* Sulz. It is subject to great variations in colour according to the season and the numerous food plants, on which it has hitherto been found feeding. During the winter it prefers to remain on the cruciferae and to return to peach, plum and nectarine with the advent of the hot weather. In India it has hitherto been recorded from Lahore [Das, 1918], Bangalore [Theobald, 1923] and South India, mostly on peach, tobacco, cabbage, cauliflower, mustard, turnip, potato, tomato, radish, Sissoo (*Dalbergia Sissoo*), Dhatura (*Datura stramonium*), nasturtium (*Tropœleum officinale*), etc. It has also been reported from Japan, Australia, North America, South America, Africa, most of the European countries, Bermuda, Java, and Hawaii on a great variety of food plants and has been reported to be a carrier of potato mosaic [Smith, 1927] potato crinckle [Hughes, 1930], tobacco mosaic [Smith, 1931], as an agent in virus transmission [Hogan, 1929], and as a possible vector of "Breaking" in tulip species [Hughes, 1930]. In a recent paper Smith [1931] has stated it to be a carrier of fourteen kinds of virus. As no critical study has been made of the pest under Indian climatic conditions, it is not possible to incriminate it as a carrier of any of the virus diseases.

The infested trees were treated with crude oil emulsion at one pint to four gallons of water, rosin compound 1 in 5, tobacco decoction and tobacco dust, ground very fine. It was then found that the aphis was particularly susceptible to the action of nicotine preparations. The effect of spraying with nicotine preparation was quick and effective, and as such it was used exclusively to deal with the sudden outbreak of the pest. Tobacco solution was made by soaking two pounds of strong, tobacco dust of the local *Kauniya* variety, easily procurable in the vicinity of Pusa, in four gallons of water for tweny-four hours, occasionally stirring the solution and then straining it through a muslin cloth, soft soap, at the rate of a pound to twenty gallons of the spray fluid, was added to increase the wetting

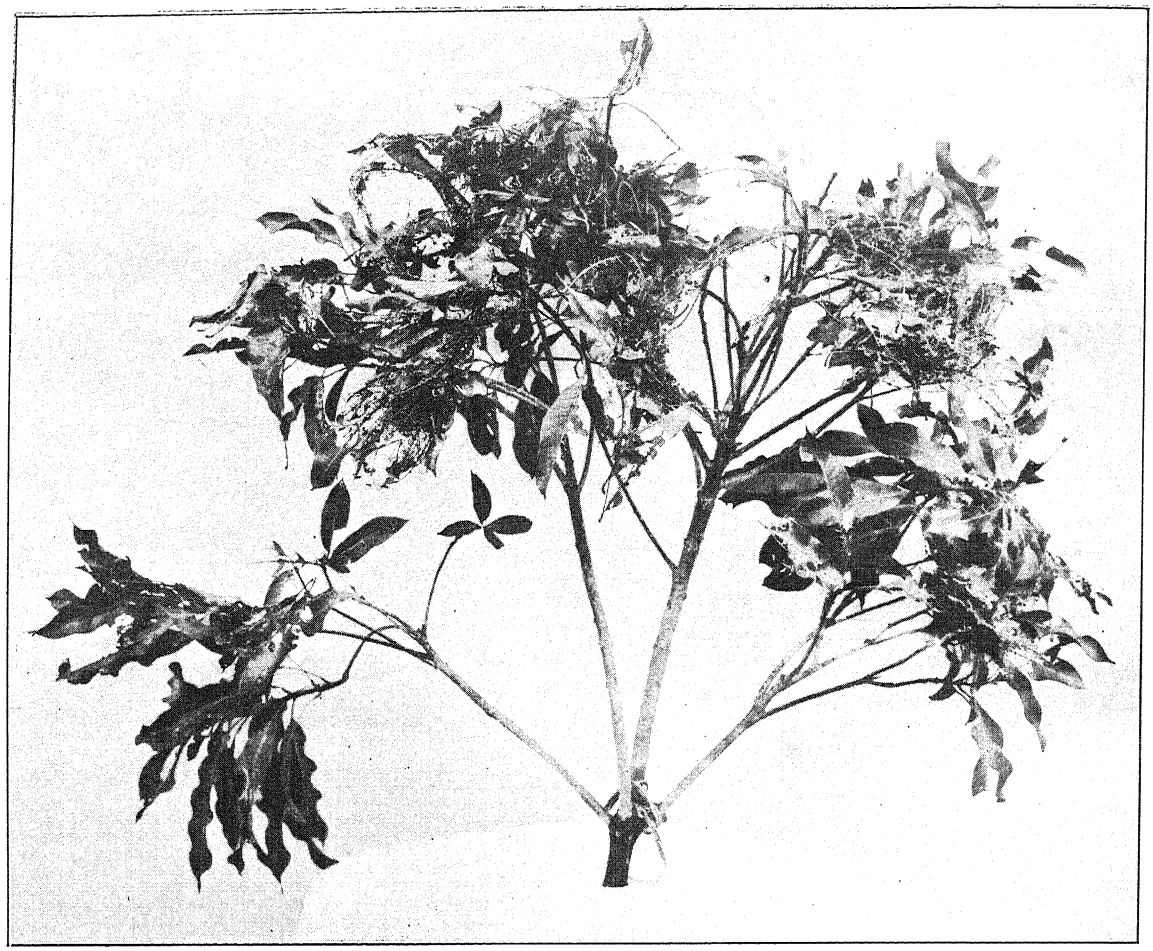


Fig. 1.

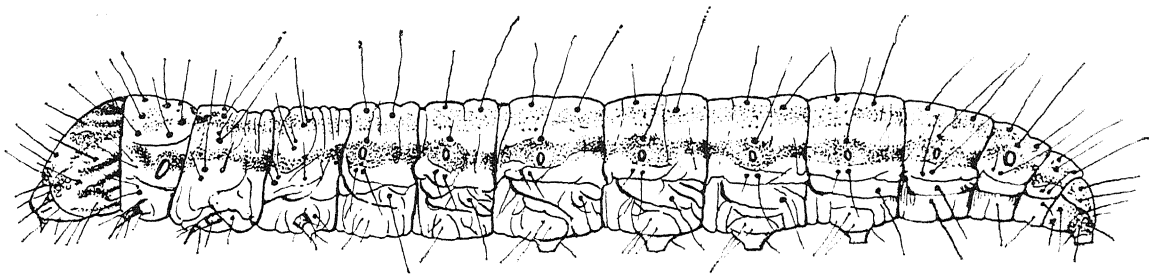


Fig. 2.

(For explanation please see page 541.)

power of the spray fluid, and the result was that the infested trees were clear of the pest within twelve hours of the first application. The average cost of spraying ten medium sized peach trees was a little over an anna per tree.

	a.	*p.*
Insecticides	6	8
Labour	4	0
	10	8

In order that spraying with tobacco solution be effective it is necessary to spray the infested trees before the leaves begin to curl up, as once the nymphs and the adults have established themselves within the folded leaves it is not only difficult, but expensive also, to dislodge them, and this could only be done with a force-pump which is costly. It was also noticed that the finer the tobacco dust the more effective it was against the wingless viviparous females and nymphs on the leaves and shoots of peach trees.

This short note is intended to record the serious nature of the injury brought about by a pest which remained dormant in the locality previously, broke out suddenly, did the maximum amount of damage to fruit trees in the minimum of time and disappeared as suddenly as it had appeared. In fact, the very presence of *Myzus persicae* Sulzer, was not recorded in North Bihar prior to its outbreak in 1930. Its sudden appearance, however, corresponds more or less to that of *Nephotettix bipunctatus* Fabr. and *Nephotettix apicalis* Motsch. on paddy in the Central Provinces (full details of which have already been given in *Memoirs Dept. Agric. India*, Volume V, No. 5, May 1920, and Bull. No. 104 *Agric. Res. Inst. Pusa*, 1921) and *Orthaga mangiferae* n. sp. which broke out last year, did considerable damage to the grafted mango trees in the neighbourhood of Pusa from August till November 1931 (Plate LVIII, fig. 2). The caterpillars appeared in swarms, defoliated the trees and disappeared by the middle of November. They were robust and mottled dusky-grey in appearance. Each caterpillar is 27 mm. long, greatest breadth over thorax 3·5 mm., pale, mottled fuscous, with a broad, chitinous head mottled with black, irregular, dark-grey fuscous lines. A pair of dark, fuscous longitudinal lines run dorsally from pronotum to anal end; a broad, olivaceous longitudinal line dorso-medianally from mesonotum to anal end, with a pair of tubercles ending in brown setae, irrorated at base with black on either side of the medio-dorsal longitudinal line; posterior end incurved with a number of brown, porrect setae. The full-fed caterpillars are plump and active. They feed gregariously on the tender top leaves which they web together. The infested tree soon becomes stripped of its foliage and is covered over densely with a net work of webs, within which the caterpillers pupate (Plate LVIII, fig. 1).

The adult moth *Orthaga mangiferae* n. sp. is 30 mm. in expanse, with head and thorax brownish grey; forewing suffused with brownish olive-green; a black spot at base of cell, with dark-fuscous tufts of scales at end of cell; a dentate, post medial dark line with whitish outer edge excurved from costa to vein 3 and then incurved; a marginal series of dark specks; celia greyish rufous. Hind wing fuscous brown with a slightly waved curved post-medial line. [Plate LIX, figs. 1-5]

Full details of life-history are not known as reports regarding its outbreak were received late in the season. The pest is still under observation and a full account will be given hereafter when details of life-history and treatment have been fully worked out.

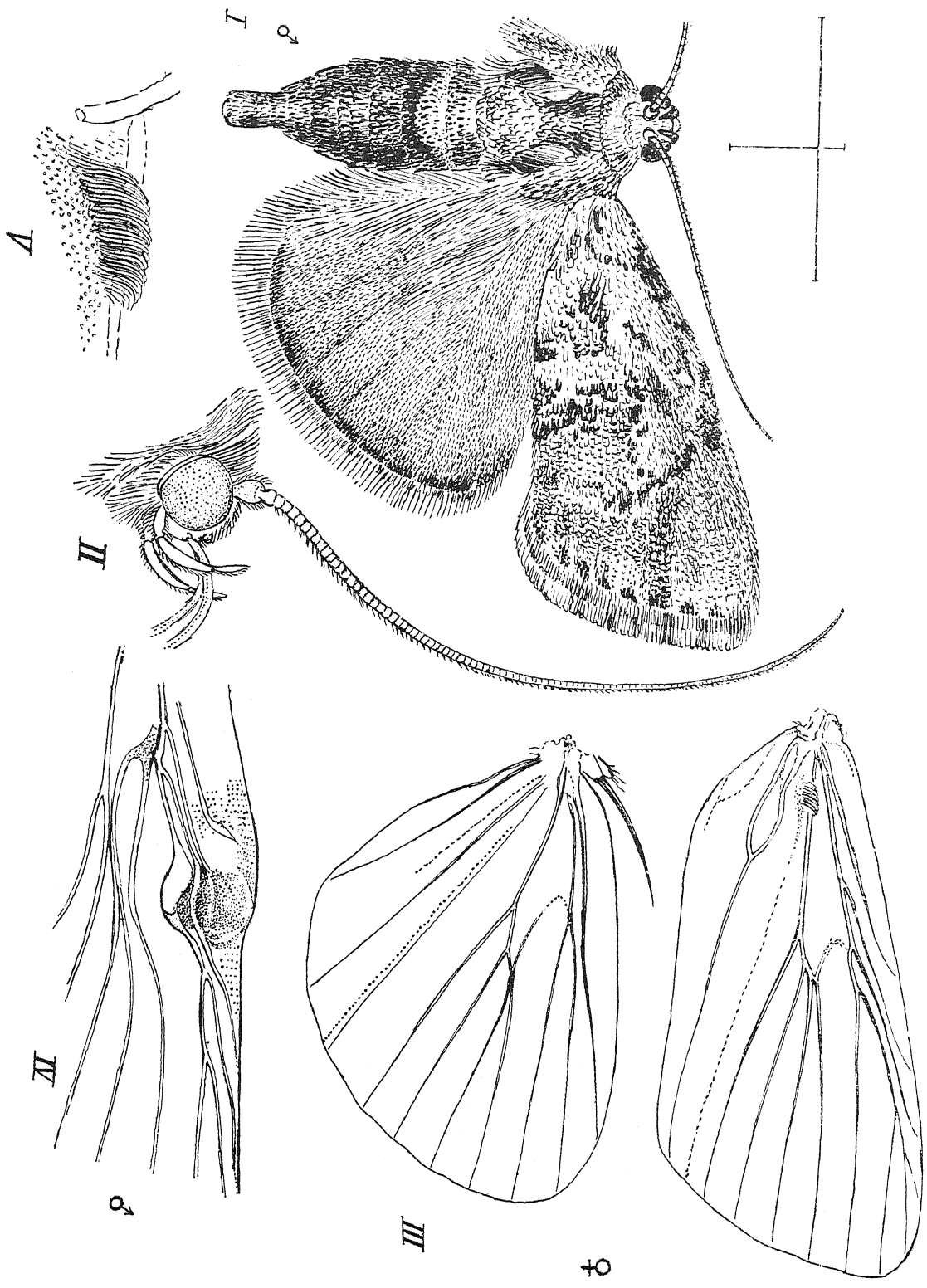
REFERENCES.

Baker (1920). *Calif. Monthly Bull.* **9**, 203.
Cayley (1928). *Ann. Appl. Biol.* **15**, 529.
Das (1918). *Mem. Ind. Mus.* **6**, 166.
Gaumont (1923). *Phytopathology & Econ. Entom.* 665.
Goot (1915). Beitrage zur Kenntnise der Hollandischen Blattlause, 170.
Gurney (1924). *Agric. Gaz.* N. S. W. **35**, 667.
Hardenburg (1913). *Agric. J.* U. S. A. **6**, 228.
Hogan (1929). *Phytopathology* **19**, 109.
Hossfall (1924). *Pennsyl. Agric. Expt. Stat. Bull.* 185.
Hughes (1930). *Ann. Appl. Biol.* **17**, 36.
Koch (1849). *Die. Pflanz.* **42**, 55.
Pailot & Fauve (1923). *C. R. Acad. Agric. France* **9**, 661.
Schrank (1801). *Fn. Boica.* **2**, 114.
Smith (1927). *Ann. Appl. Biol.* **14**, 130.
——— (1929). *Ann. Appl. Biol.* **16**, 1.
———(1931). *Biol. Reviews* **6**, 302.
Sulzer (1776). Abgek. Gesch. Ins. 105.
Taylor (1908). *J. Econ. Ent.* **1**, 83.
Theobald (1923). *Ento. Month. Mag.* **59**, 91.
——— (1926). *Aphididae of Great Britain* **1**, 318.
Zeck (1928). *Agric. J.* N. S. W. **39**, 147.

EXPLANATION TO PLATES.

Plate LVII, Fig. 1 . Epidermal puncture by *Myzus persicae* in the neighbourhood of a vein. The puncture traverses almost the entire diameter of leaf; Xylem and phloem tapped. Tracheide filled with saliva . . . ×800 [After Smith

,, Fig. 2 . Epidermal puncture by *Myzus persicae* showing collapse of epidermal cells and destruction of cell contents . . . . . ×650 [After Smith



(For explanation please see page 541.)

Plate LVIII, Fig. 1 . Top portion of three year old grafted mango tree damaged by *Orthaga mangiferae*, n. sp. caterpillars . . . . . [Original

„ Fig. 2 . Full fed larva of *Orthaga mangiferae* . . . side view [Original

Plate LIX, Fig. 1 . Adult moth ♂ . . . . . ×6

Fig. 2 . Adult moth ♂ side view of maxillary palpi and antenna . . . . . much enlarged

Fig. 3 . Fore and hind wings ♂ . . . . . ×8

Fig. 4 . Glandular lobe on costa of forewing ♂ . . . much magnified.

Fig. 5 . Retinaculum with jugum ♀ . . . . . much magnified.

WOODHOUSE MEMORIAL PRIZE, 1932.

In memory of Mr. E. J. Woodhouse, Late Economic Botanist and Principal of Sabour Agricultural College who was killed in action in France in 1917, a prize in the form of a silver medal and books of a combined value of Rs. 85 will be awarded to the writer of the best essay on a subject of botanical interest to be selected from the list noted below. The length of the essay should not exceed 4,000 words.

The competition is open to graduates of Indian Universities and to Diploma holders and Licentiates of recognised Agricultural Colleges in India who are not more than 30 years of age on the date of submission of their essays.

Papers should be forwarded to the Director of Agriculture, Bihar and Orissa, Patna, before November 1st, 1932.

Failing papers of sufficient merit no award will be made.

D. R. SETHI,
Director of Agriculture, Bihar and Orissa.

List of subjects for 1932 prize :—

1. The economics of an agricultural holding in North Bihar.
2. A scientific basis for the classification of the varieties of rice cultivated in India.
3. Factors governing yield of crops.
4. The importance of root studies in crop improvement.

ORIGINAL ARTICLES

LIFE-HISTORIES OF SOME INDIAN SYRPHIDAE

BY

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AND

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(Received for publication on the 27th April 1932.)

(With Plates LX-LXVII.)

INTRODUCTION

The Syrphidae of India, as far as the adults are concerned, have been dealt with by Brunetti [1923]. Since then no attempt has been made to revise his work on the family. Taking Brunetti's work as a basis for further investigations, such a revision is badly needed, for it will bring forth some very useful information and add many new genera and species to the list of those already described in the Fauna Volume. There is a great diversity of forms within this family and there is no family of insects which resembles bees and wasps so much as the Syrphidae. Like the adults the larvae of Syrphidae present different forms associated with different feeding habits. Some are of the ordinary maggot type, others possess a long tail which is three to four times the length of the body and on this account have been called rat-tailed larvae, and still others (*microdon*) possess such a peculiar combination of characters as to have baffled the entomological workers so much as to have made them give to the larvae the rank of distinct species in the Phylum Mollusca.

The economic status of the family is on the whole beneficial. There are some species the larvae of which have been known to cause some damage to the leaves and flowers of maize [Riley and Howard, 1888] in certain localities in America and still others, *e.g.*, *Eumerus strigatus* whose larvae destroy to a certain extent the bulbs of onion. Verrall [1901] mentions that *Eumerus strigatus* Fallen has been bred from the bulbs of common onion (*Allium cepa*) of which they sometimes destroy the whole crop. The larvae occurred in July and pupated in the bulbs or in the neighbouring earth; sometimes bred from soft and rotten bulbs. These stray instances of damage done by the larvae of some species of Syrphidae are nothing as compared to the immense good done by others the larvae of which prey upon the nymphs of Aphidae, Coccidae, Psyllidae and Aleyrodidae. The ravages of plant lice, scale insects and the white fly to the cultivated crops are too well known, both to the cultivator and the scientific worker, and therefore need not be mentioned here.

It seems that the good done by the larvae of the aphidophagous species of Syrphidae in reducing to a considerable extent the attack of plant lice has not been properly appreciated, for, had it been so, our knowledge of the immature stages of the species of these flies would not have been so incomplete. Excepting a plate illustrating the various stages in the life-history of *Ischiodon scutellaris* (Fabr.) in the Indian Insect Life [Howlett, 1910] and some notes on *Paragus serratus* (Fabr.) published in *Pusa Bull.* No. 59 [Fletcher, 1916] there is no published record of the immature stages of any other species of Syrphidae feeding on Aphids.

The present paper is the result of observations made on the various stages in the life-cycles of some aphidophagous species both in the field and the laboratory during the years 1931-32, and has been written with the intention of helping the scientific worker with some information which may be of some use to him in distinguishing in the field the larvae of the various species dealt with in this paper.

The life-histories of altogether eight species have been included in this paper, of which seven are those of the aphidophagous species and the eighth deals with the life-history of *Helophilus bengalensis* (Wied.). The life-history of each species is accompanied by a plate. Some of the drawings in the plates have been made by the senior author and hence are not artistic though in the matter of details they will not be found lacking.

The identification of a species of insect by its larval characters is important and desirable, but it will be found that very little attention has been paid towards this side of entomology. An attempt would have been made to draw out a table for the identification of the aphidophagous species of Syrphidae by their larval characters, had the material been enough. As such, it is hoped that the characters of the larvae of various species given here will be made use of by any future worker on this problem.

The authors wish to acknowledge their sincere thanks to their chief, T. Bainbrigge Fletcher, Esq., R. N., F. L. S., F. E. S., F. Z. S., and P. V. Isaac, Esq., B. A., M. Sc., D. I. C., Second Entomologist (Dipterist), for the keen interest that they took in the work while it was in progress. To Mr. N. Sen, the artist of the Entomological Section, they are very grateful for most of the illustrations in the plates.

The relation between the larvae of aphidophagous species of Syrphidae, their hosts and the plants on which they are found

There are many plants both wild and cultivated which are infested by one or more specis of aphids, and some are heavily attacked. Besides this there are insects belonging to other families such as Coccidae, Aleyrodidae, Psyllidae, Membracidae and Jassidae, the nymphal stages of which do appreciable damage by sucking the juices of the plants upon which they are found. Very often larvae of a single

species of Syrphidae or belonging to two or three distinct species will be found among the colonies of aphids on a plant. It is rare to come across a colony of aphids on a plant which does not possess in the midst of its individuals a Syrphid larva. The Syrphid larvae prey upon the aphids very voraciously, so much so, that sometimes the plant is free from their attacks. The voraciousness of the Syrphid larva can not be questioned. A simple observation with a pocket lens in the field while the larvae are feeding, or in the laboratory when they are brought for examination will prove this. It was observed in a four to five days old larva of *Baccha pulchrifrons* Aust., which was found feeding on the nymphs of the Psyllid, *Ctenophalara elongata* Crawf., that it sucked a nymph of small size in less than a minute after which it attacked another bigger in size and sucked it in one minute and thirty seconds. The larva was then placed in a Petrie-dish with a leaf of *Bombax malabaricum* infested with the nymphs (both grown up and small) of *Ctenophalara elongata* Crawf. Only one hundred nymphs were allowed to remain on the leaf and the rest removed. The larva was examined after an hour and was found to have killed sixty nymphs. Similar observations were made on larvae of *Paragus serratus* (Fabr.), *Sphacrophoria javana* (Wied.), and *Syrphus balteatus* (De Geer) and the results obtained were much the same as derived from the larva of *Baccha pulchrifrons* Aust.

The larvae of the aphidophagous species of Syrphidae take in more food than is required for enabling them to pupate. This surplus amount of food is stored by them in the form of fat. The amount of fat stored by the larva is in proportion to the quantity of food available to the larva in the field. Thus the larvae possess sufficient powers to resist starvation. Two adult larvae of *Baccha pulchrifrons* Aust. bred under laboratory conditions with ample food were found to resist starvation for a number of days, one dying nine days, and the other ten days, after they were subjected to these conditions.

It is quite natural to presume that a close relationship exists between the life-cycle of a particular species of Syrphidae and that of the aphid upon which it preys. This statement was verified during field observations, when many times a single newly hatched Syrphid larva was found on the lower side of the leaf of the plant in association with very few aphids thus proving that the larvae of the aphidophagous species or Syrphidae make their appearence just at the time when the aphids begin to form colonies. They complete their life-cycle while the aphids are on the plant and disappear with the disappearance of the aphids. Nature has therefore introduced enemies to check the prodigious production of the aphids and has saved the plants from destruction.

The larvae of the aphidophagous species of Syrphidae are very voracious. A Syrphid larva devours within a period of one to two weeks of its existence a good

lot of aphids thus saving the plant from its worst enemy during the most critical period of its growth. Although the benefit derived by the plant may not in some cases be appreciable on account of the attack of the aphids being serious and the plant may show signs of withering, yet it may be borne in mind that the harm done by the aphids would have been incalculable if they would have been allowed to breed unchecked. If, as Reaumour calculated and others have substantiated, one aphid may be the progenator of over 5,000,000,000 individuals during her existence of a month or six weeks, one can imagine how much benefit the plant derives from these natural enemies of the aphids.

The eggs of the aphidophagous species of Syrphidae are laid singly on the surface of the leaves. The eggs are chalk white and can easily be recognized on the green surface of the leaf on which they are laid. They are seen laid during the day time when the flies are seen actively hovering over the plants, searching for suitable places for laying eggs. Invariably a limited number of eggs are seen on a leaf. Usually one or two eggs or at the most five eggs have been observed on a leaf infested with aphids. An egg will be found very near a colony of aphids, so that on hatching it may not have to search for food which will be lying very close to it. It will be found that a distinct relation exists between the number of aphids and the number of Syrphid larvae that are to prey upon them on a plant. The interest of the plant is also concerned, for, if the aphids are in a relatively greater proportion to the Syrphid larvae, there is a danger of the plant being seriously damaged, and if they are in a lesser proportion, there is a danger of Syrphid larvae dying of starvation.

A peculiar case of predatism was recorded while studying the life-history of *Baccha pulchrifrons* Aust. The egg of this species are laid in association with the nymphs of the Psyllid, *Ctenophalara elongata* Crawf., on the leaves of *Bombax malabaricum*. On the same leaves the stalked eggs of a species of *Chrysopa* (which unfortunately could not be determined) are also laid. The eggs of the *Chrysopa* are light greenish-yellow when they are freshly laid, and turn brown at the time of hatching. The larvae hatching from these eggs are reddish purple at the time of maturity. They are provided with long legs, very long spines on the abdominal segments and powerful inwardly curved hollow mandibles. The *Chrysopa* larva is a very voracious feeder on Psyllid nymphs. Not only that, it was also found to prey upon the eggs and larvae of *Baccha pulchrifrons* Aust. It breaks open the egg shell by the sharp ends of its mandibles after which it sucks the contents of the egg. A single larva of *Chrysopa* was confined in a cage with four larvae of *Baccha pulchrifrons* Aust. at 11 a.m. on 10th December 1931. Next day one larva of the latter was found completely sucked and another partially so by the former which was then in the act of forming a cocoon. In order to find out whether the larvae of *Baccha pulchrifrons* Aust. also prey upon the eggs of *Chrysopa*, two seven-days-old larvae of

the Syrphid fly were placed with 17 eggs of the *Chrysopa* and some Psyllid nymphs in a cage on 11th December 1931. The Syrphid larvae were closely watched while feeding, and during these observations the larvae were found to bend the stalks so as to bring down the eggs on the surface of the leaf and then suck them up. Therefore, there exists a keen struggle for existence between the larvae of *Chrysopa* and those of *Baccha pulchrifrons* Aust., the former preying upon the eggs as well as the larvae of the latter which only preys upon the eggs of the former. The loss sustained by the Psyllid colony through the attacks of the larvae of the Syrphid and the *Chrysopa* species is to a great extent minimized by their mutual warfare. The plant is also saved in a way from the attack of the Psyllid, which would have been heavy, had not there been two different kinds of larvae predating upon the nymphs of the Psyllid.

Enemies

The parasitic insects belonging to the family *Ichneumonidae* seem to be the natural enemies of the larvae of the aphidophagous species. Specimens of *Bassus multicolor* Grav., were reared from the larvae and pupae of *Ischiodon scutellaris* (Fabr.). The parasite probably drops its eggs into the body of the larva of *Ischiodon scutellaris* (Fabr.) through the skin. The larva of the parasite grows in the body of the Syrphid larva without hindering its pupation. The parasite emerges through a slit in the anterior broad rounded portion of the pupa. The emergence of the parasite takes place ten to fourteen days after the pupation of the larva. Only one parasite was seen to emerge from one pupa. The description of the adult parasite will be found in the Fauna of British India, Hymenoptera, Vol. III., pp. 279-280.

Table I

The habitats and the time of occurence of the larvae of the aphidophagous species of Syrphidae, the records of which are available in Pusa collection

Name of the species	Found feeding on	Time of occurrence	Locality
Baccha sapphirina (Wied.). .	Indigo Psylla . .	30th October 1912	Pusa
Do. ditto . . .	Orange Aphis . .	16th June 1913	Do.
Do. *pulchrifrons* (Aust.) .	*Ctenophalara elongata* Crawf. on *Bombax malabaricum*	18th February 1916	Do.
		20th February 1916	
		4th January 1927	
		15th November 1931	
		27th November 1931	
		5th December 1931	

Name of the species	Found feeding on	Time of occurrence	Locality
Paragus serratus (Fabr.)	Probably bred on root aphis	19th September 1913	Coimbatore
Do. ditto	Aphis on red gram shoots	23rd November 1906	Samalkot
Do. ditto	Green aphis on *Phyllanthus emblica*	27th July 1914	Pusa
Do. ditto	Aphis on *Solanum* sp.	20th June 1915	Do.
Do. ditto	Aphis on water-melon	30th May 1907	Hagari
Do. ditto	Aphis on *Dolichos Lablab*	9th May 1931	Pusa
Ischiodon scutellaris (Fabr.)	Aphis on *Solanum* sp.	19th November 1915	Do.
Do. ditto	Aphis on Chrysanthemum.	28th January 1915 30th January 1915 1st November 1915	Do.
Do. ditto	Aphis on water-melon	2nd May 1907 29th May 1907	Hagari
Do. ditto	Aphis on Ak (*Calotropus*)	2nd June 1906	Pusa
Do. ditto	Aphis on cotton	3rd July 1906 2nd August 1906 15th February 1908 2nd March 1908 1st September 1904	Do.
Do. ditto	Aphis on cabbage	27th April 1905 25th March 1908	Do.
Do. ditto	Wheat aphis	7th March 1908	Do.
Do. ditto	Aphis on sisso	17th May 1931	Do.
Do. ditto	Aphis on mustard	23rd February 1932 1st March 1932 16th March 1932	Do.

Name of the species	Found feeding on	Time of occurrence	Locality
Sphaerophoria javana (Wied.) .	*Ctenophalara elongata*, Crawf. on *Bombax malabaricum*	7th November 1931 23rd November 1931	Pusa
Do. ditto . . .	Aphis on cotton . .	30th November 1931 23rd December 1931	Do.
Syrphus serarius (Wied.) . .	Mustard aphis . .	4th March 1932 8th March 1932	Do.
Do. *balteatus* (De Geer) .	Aphis on sunflower .	25th February 1907	Do.
Do. ditto . . .	Aphis on cotton . .	20th December 1931	Do.
Do. *confrater* (Wied.) .	Cabbage aphis . .	16th March 1908	Do.
Do. ditto . . .	Aphis on chrysanthemum	1st February 1915	Do.
Do. ditto . . .	Wheat and cotton aphis .	14th March 1908	Do.
Do. ditto . . .	*Eriosoma lanigera* (Woolly aphis).	21st September 1923	Srinagar (Kashmir)
Do. ditto . . .	Pomegranate aphis .	25th September 1923	Do.
Do. *isaaci* sp. nov. . .	Aphis on mustard .	7th February 1932 11th February 1932	Pusa

LIFE-HISTORY OF *Baccha pulchrifrons* AUSTEN

Introduction

The genus *Baccha* can easily be distinguished by the following characters :—

Head more than hemispherical, face hollowed below frontal prominence, produced again to a central knob, not produced at upper mouth edge ; species dark with pale markings on the head, thorax and abdomen, the latter conspicuously constricted at the base.

Baccha pulchrifrons Aust. has been recorded from Bhowali, Darjeeling district; Pusa, Bihar; Mormugoa, Goa; Hot Wells, Trincomalee; Ceylon; Cherrapunji, Assam and jungle at the base of Dawna Hills.

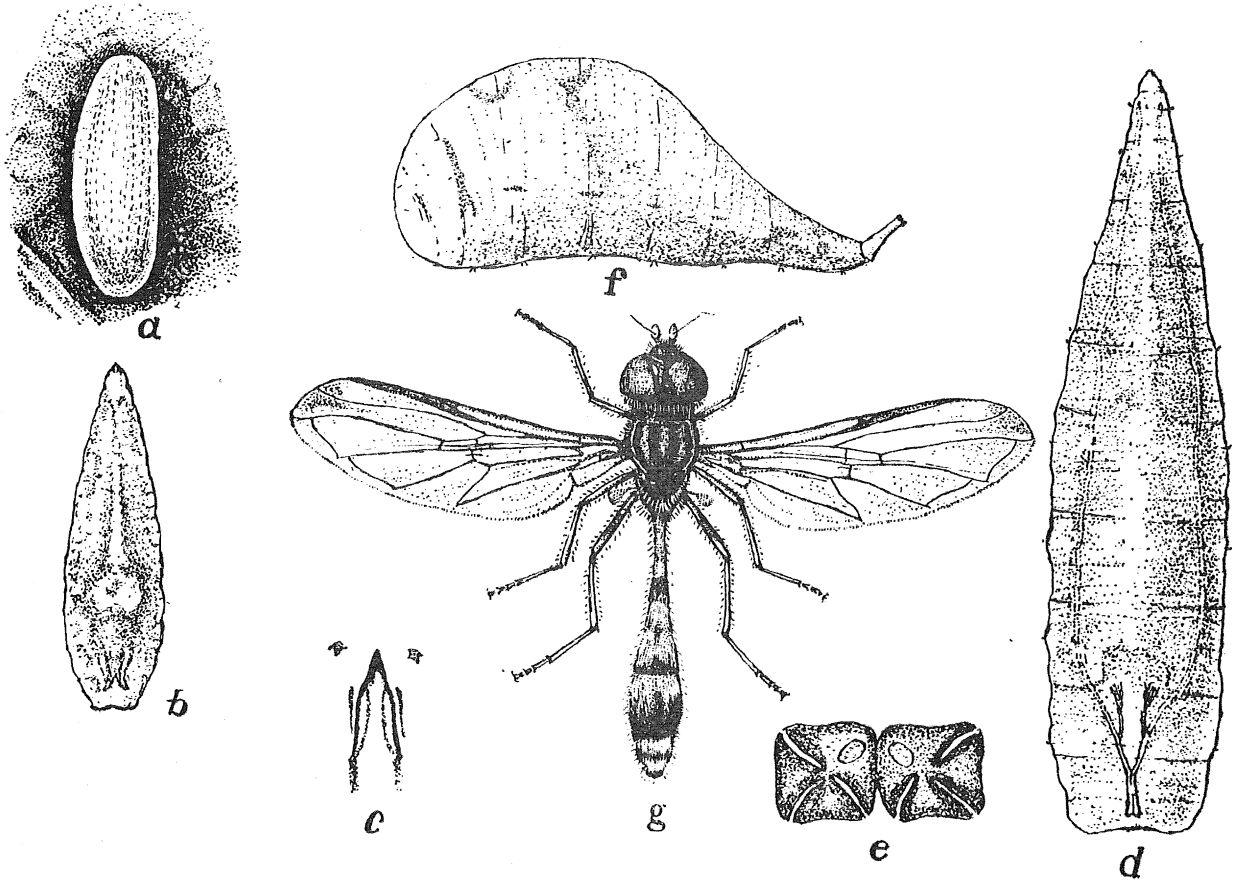
Some observations

In Pusa the fly has been collected during the months of January, February, August, November and December. About the middle of November 1931, the flies were seen hovering about the young plants of *Bombax malabaricum*, probably in search of suitable places for egg laying. It is about this time that the nymphs of the Psyllid, *Ctenophalara elongata* Crawf. make their first appearance on the leaves. The leaves of *Bombax malabaricum* infested with Psyllid nymphs were thus collected from the trees and brought to the laboratory for detecting the eggs. After a careful search the eggs were found. The nymphs of the Psyllid were found both on the upper and the lower surface of the leaves, more particularly on the lower surface as it is soft being less exposed to sun than the upper surface. If the infestation is less, the nymphs are seen collected close to the primary vein of the leaf and in case it is heavy they are seen on the secondary veins also or spreading on the entire surface of the leaf. The eggs of *Baccha pulchrifrons* Aust., as of all aphidophagous species of Syrphidae are deposited singly. An egg is laid by the fly just near a small colony of Psyllid nymphs, so that the larva on hatching may find food at once to begin its existence. The number of eggs laid by the fly is proportionate to the nature of infestation. Usually one to two eggs or at the most four eggs were noticed on a leaf.

Field collections of leaves infested with nymphs of *Ctenophalara elongata* Crawf., were made on a large scale for the eggs and larvae of *Baccha pulchrifrons* Aust. About 200 such leaves were examined every day and eggs and larvae were sorted out and kept separately in Petrie-dishes with food for rearing. Daily observations in the colour changes of the larvae and the pupae of this species were made and the life-history was thus worked out.

The egg (Plate LX, fig. *a*)—The freshly laid egg is milk-white with a light yellow tinge about its middle. It is ·90 mm. long and ·30 mm. broad. It is elongated oval in outline, rounded at both the ends, rather slightly narrower at the anterior end which bears the mycropyle on a small conical projection. The chorion of the egg is sculptured with small rod-like calcarious structures arranged in longitudinal rows parallel to the long axis of the body of the egg. Each such structure sends out very small branches on both the sides of its body. These small branches from the structures do not form a net-work.

The approximate incubation period can be judged from Table II given below which gives the date of collection and the date of hatching of the eggs.



Baccha pulchrifrons Austen.

(*For explanation please see p. 570.*)

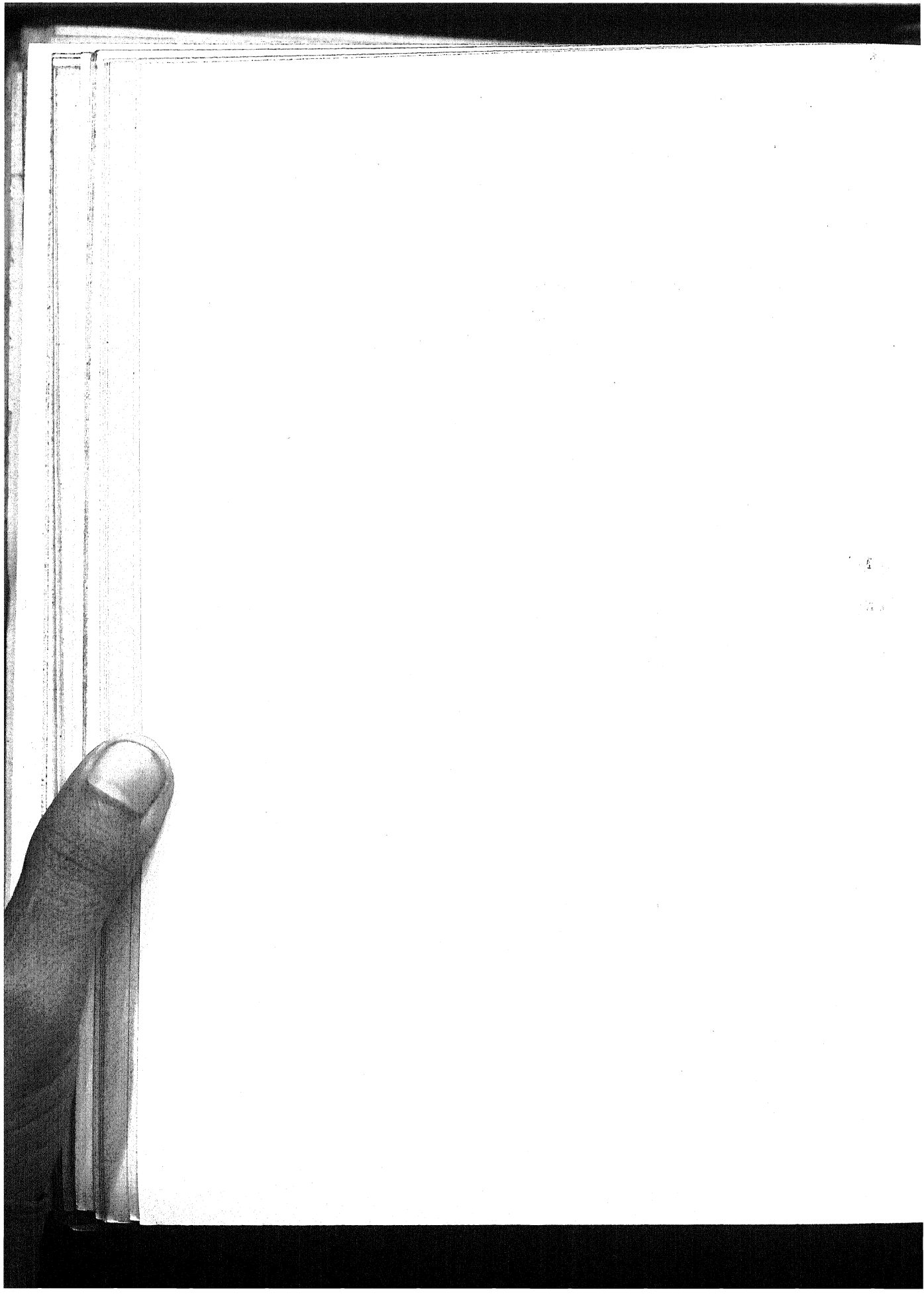


TABLE II

Giving the date of collection, the number of eggs collected and the date of hatching of eggs of Baccha pulchrifrons *Aust.*

Date of collection of eggs	Number of eggs collected	Number of eggs hatched and the date of hatching
7th November 1931 (morning) .	3	Two eggs hatched at 10 a. m. on 8th November 1931
		One hatched at 8 a. m. on 9th November 1931
13th November 1931 ,, .	1	Hatched at 9 a. m. on 14th November 1931
14th November 1931 ,, .	8	Three hatched at 1 p. m. on 15th November 1931
16th November 1931 ,, .	8	Six hatched at 10 a. m. on 18th November 1931
20th November 1931 ,, .	14	Thirteen hatched at 11 a. m. on 22nd November 1931
21st November 1931 ,, .	8	Three hatched at 8 a. m. on 23rd November 1931
23rd November 1931 ,, .	8	Four hatched at 11-30 a. m. on 24th November 1931
27th November 1931 ,, .	14	Four hatched at 10 a. m. on 29th November 1931
28th November 1931 ,, .	13	Four hatched at 9 a. m. on 30th November 1931

The young larva (Plate LX, fig. *b*)—The newly hatched larva is 1·92 mm. long and ·53 mm. broad. The body is sub-cylindrical being flat ventrally and convex dorsally. It is broad posteriorly and is seen to narrow gradually towards the anterior end. In colour, it is greyish white, and beneath the skin, yellowish about the middle and light orange towards the hind end of the body. The segments of the body are marked with transverse wrinkles in the skin at regular intervals. The oral aperture is situated ventrally at the anterior end of the head within which is the œsophageal framework containing the jaws and the mouthhooklets worked backwards, forwards and transversely by a complex set of muscles controlling the working of the œsophageal framework. The two tracheal tubes, one on each side, run a wavy course within the body. They open anteriorly at the sides of the prothoracic segment by the anterior spiracles placed at the end of the small chitinous anterior larval respiratory cornua and posteriorly by spiracles on the last

segment mid-dorsally at the ends of the posterior larval respiratory tubes which in the young larva are separate. The median dorsal blood vessel is almost colourless and can be made out by the pulsation of its various chambers. If followed from the posterior to the anterior end, it is seen to disappear at the prothoracic segment. On the sides of the dorsal blood vessel at about the anterior third of the body of the larva are two triangular patches of fat, their apices being directed forwards. Another rectangular fat area is situated just behind the middle of the body and extends upto the penultimate segment of the body of the larva. The posterior side of this area is produced downwards at the angles. Pairs of small patches of fat, one such pair in each segment excepting the last, are found dorsolaterally in the body segments of the larva.

The young larva feeds very voraciously on the young Psyllid nymphs. During the time when it is feeding, it will be seen piercing at various places the soft abdomen of its victim by the pointed anteior portion of its jaws. As soon as the punctures are made, the jaws begin to work again vigorously backward and forward and by their action, aided by the mouth hooklets, the victim is sucked up completely, the remains of its body being left on the leaf as a small crumpled piece of chitin. It goes on feeding upon its prey in this manner for some time after which it rests on the surface of the leaf during which the pulsation of the various chambers of the heart can very well be seen. It soon becomes active after the repose taken and begins attacking other nymphs on the leaf.

In Pusa the larvae of *Baccha pulchrifrons* have only been found feeding on *Psylla* on *Bombax malabaricum*, there being no alternative hosts. The larvae were tried under laboratory conditions on cotton aphis. Two larvae of *Baccha pulchrifrons*, about six to seven days old, were kept in a cage with all sizes of nymphs of cotton aphis on a leaf of cotton. Fresh food was given to the larvae every day. Although the food was so near at hand, the larvae did not even go near it and were ultimately starved to death, one dying 10 days, and the other 11 days, after they were kept on this food.

Full-grown larva (Plate LX, fig. *d*).—The full-grown larva of *Baccha pulchrifrons* is 8·5 mm. long and 2·0 mm. broad. It is of a light grey colour with a prominent rectangular white fat area on the dorsal side just behind the middle of the body. The head of the larva is apparently composed of two segments. Protruding from the tip of the first segment at the time of feeding of the larva, can be seen the jaws and the anterior mouth hooklets. The antennae are seen to arise ventrally from the first segment of the head. Each antenna is bifurcated at its distal end, the inner piece being composed of two joints. The first segment of the head is covered over with small sensory spines. In each segment of the body excepting the prothoracic and the last there are three false lines due to wrinkles in

the skin. On account of these wrinkles it is rather difficult to ascertain the number of segments in the body of the larva. The arrangement of spines at regular distances in the region of the body of the larvae of most of the aphidophagous species of Syrphidae solves the difficulty by giving an indication of the position of inter-segmental lines. These spines have been named as segmental spines, but we should prefer to call them inter-segmental spines as they are situated in the inter-segmental regions of the body of the larva. There are 10 rows of spines in the larva of *Baccha pulchrifrons* as in other larvae of the aphidophagous species of Syrphidae. The first row of spines is between the head and the prothoracic segments and the last row between the penultimate and the last segment. In each row there are 12 spines, *viz.*, a pair of median, a pair of dorsal, a pair of dorso-lateral, a pair of ventro-lateral and 2 pairs of ventral spines. Besides this, there is a small bristly pubescence all over the body of the larvae of most of theaphidophagous species.

These inter-segmental spines are more prominent in the young larva. In the adult larva they can only be made out with the help of a microscope. Each spine has the basal portion and the tip white, the middle portion being black.

The mouth parts of the larva (Plate LX, fig. *c*) consist, as in the larvae of other aphidophagous species, of a pair of inverted V-shaped jaws and 2 pairs of mouth hooklets (2-4 pairs in other species). All these parts are heavily chitinized and are of a black colour. The two pairs of mouth hooklets are an outer and a lateral pair. The hooklets of the outer pair are bidentate, those of the lateral pair are rod-like and slender.

The two tracheal tubes are clearly seen through the translucent skin of the larva. Anteriorly they open by spiracles placed at the tips of the two anterior larval respiratory cornua, one on each side of the prothoracic segment and posteriorly each tube is continued separately into the chitinous respiratory tube situated on the mid-dorsal portion of the last segment. The chitinous respiratory tubes which are separate in the younger stages of the larva become closely applied by their inner sides and form a single posterior respiratory appendage which is seen prominently sticking out from the last body segment. In the adult larva the posterior respiratory appendage is about 1 mm. long. Each tracheal tube gives a branch on its inner side before it passes into the respiratory appendage. This branch is divided at its end into fine braches supplying the ninth segment. At the end of the respiratory tube on each side are seen three digitate spiracles at the three angles and at the fourth inner angle a dorsal circular plate as shown in Plate LX, fig. *e*.

The two anterior triangular fat areas of the young larva cannot be seen in the adult larva. The two small dorso-lateral patches of fat in each segment are clearly

seen in the full-grown larva. The posterior rectangular fat area is very prominent and extends over the ninth, eighth and seventh segments before which it is seen narrowed abruptly up to the fourth segment.

The dorsal blood vessel is of a light yellow colour in some specimens and in others it is almost colourless and can be made out by the pulsation of its various chambers. It can be followed from the ninth to the fourth segment.

Table III below gives the date of hatching and the pupation of the larvae. From it the larval period can easily be found out.

TABLE III

Showing the larval period in Baccha pulchrifrons *Aust.*

Larva No.	Date of hatching of the larva	Date of pupation of the larva	Larval period
	1931	1931	days
1	9th November . . .	20th November . .	11
2	15th ,, . . .	27th ,, . .	12
3	22nd ,, . . .	7th December . .	16
4	22nd ,, . . .	9th ,, . .	18
5	23rd ,, . . .	7th ,, . .	15
6	24th ,, . . .	10th ,, . .	17
7	29th ,, . . .	11th ,, . .	13
8	30th ,, . . .	11th ,, . .	12

The shortest larval period under laboratory conditions is 11 days and the longest 18 days. The lengthened larval period of *Baccha pulchrifrons* in some cases may partly be due to the varying conditions in temperature and humidity under which these larvae were reared and mostly to the insufficient food supply.

Pupa (Plate LX, fig. *f*)—Length 5·6 mm., breadth 2·15 mm., moulting has never been observed in the larvae of Syrphidae. The tough pupal skin is made by the induration of the larval skin of the adult larva.

The pupa of *Baccha pulchrifrons* is of a light straw colour, and like the pupae of all the aphidophagous species of Syrphidae is broad and rounded anteriorly, and tapering gradually towards the posterior end. The ventral surface is flat, the dorsal prominently convex. In a newly formed pupa many of the larval details *e. g.*, the posterior fat area, the paired dorso-lateral patches of fat, the spines and the wrinkles in the body segments, can well be seen. The spines in the pupa are very small.

The segmentation is indistinct. Dorsally about the middle of the body of the pupa are two small dark diamond shaped areas. There are also five pairs of narrow black markings in the dorso-lateral portion of the pupa. A pair of narrow black transverse stripes is seen in the broad rounded anterior part of the pupa. The ventral side of the pupa is of a straw colour.

In some pupae obtained from larvae reared in the laboratory, besides the characters mentioned above, the colour is more black dorsally due to the presence of small black dots, and a black longitudinal stripe on each side arising from which are seen four smaller backwardly directed stripes. The ventral side of such a pupa was of a light straw colour with small black dots on the sides. It was observed while rearing the larvae in the laboratory that in some cases when the larva was supplied with more food, it developed more black colour and also the dorso-lateral black stripes and the black dots. It may be mentioned here that the difference in the colouration of the pupa has no bearing on the sex of the imago. Both males and females were seen to emerge from the black type of pupa.

The pupal period is generally 11 days but flies were seen in some cases to emerge 8 or 9 days after the pupation of the larva. The pupa at the stage when the fly is about to emerge shows a red colour in the broad anterior portion—the colour of the eyes of the developing imago within the pupa case, the rest of the pupal skin appears darker, more specially the middle portion.

Imago (Plate LX, fig.*g*)—A large number of flies, both males and females, were reared in the laboratory from eggs which were collected in the field. Brunetti, in the Fauna of British India, Vol. III, pp. 123-124, while recording descriptions of male and female of *Baccha pulchrifrons* adds "Austen notes a male and female which may be a variety of this species". There are also in the Pusa collection two females of *Baccha pulchrifrons* Aust., determined by Brunetti. The males and females obtained from the eggs were compared with the determined specimens in our collection and the description given in the Fauna Volume. From the comparisons thus made, it is evident that Austen's descriptions of the male and female of *Baccha pulchrifrons* are of one and the same species.

Life-history of *Paragus serratus* (Fabricius)

Paragus serratus (Fabr.) is a widely distributed species in the east. It is also quite common in Africa. It is a small fly, 5 mm. in length with a yellow face, shining blue black punctulate thorax, with a pair of grey diverging stripes on the dorsum and with light brown to black abdomen. It can be recognised at once by its serrated scutellum. The fly is quite common in Pusa and has been collected in every month of the year. As seen from the Table given on p. 548, this species predates on root aphis, red gram shoot aphis, aphis on *Phyllanthus emblica*, aphis

on *Solanum* sp., aphis on water-melon, aphis on *Dolichos*, *Aphis sacchari*, aphis on cotton, aphis on mustard and others. It is not uncommon to see, on any one of the above-mentioned plants attacked by aphids, small greenish yellow larvae, each with a broad black stripe on the dorsal surface. Field collections of the eggs and larvae of this species were made from the leaves of *Dolichos Lablab* on which a heavy attack of aphids was noticed in May, 1931 and the life-history was worked out.

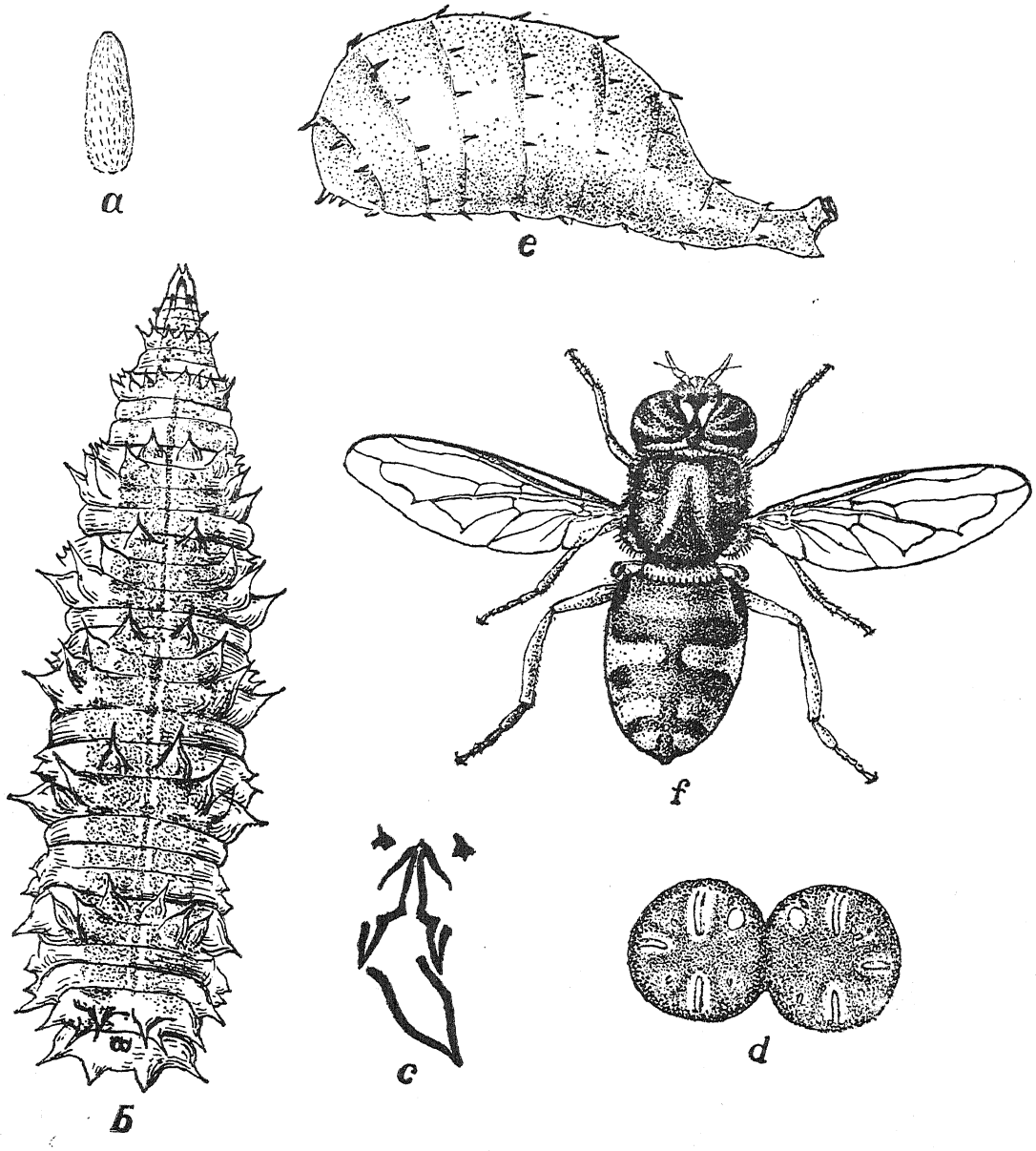
Egg (Plate LXI fig. *a*)—The egg when freshly laid is chalk-white and is ·8 mm. long and ·27 mm. broad. It is rounded at both ends, the anterior end being slightly narrower than the posterior end. The surface is ornamented with small elongated white areas arranged in longitudinal rows, each such area giving small branches on the sides.

Full-grown larva (Plate LXI, fig. *b*)—The adult larva is 9·3 mm. long and 2·5 mm. broad. The body of the larva is pale greenish yellow ventrally, black dorsally, excepting the sides (which are also greenish yellow), with splashings of orange colour throughout. The body is almost equally broad for about two-thirds of its entire length from the posterior end and is then seen to gradually narrow towards the anterior extremity. The inter-segmental spines are very prominent in this species. The bases of the spines are broad and swollen and are pale green excepting those of the median and the dorsal spines which are orange. The skin of the larva is tough and therefore the trachael tubes cannot be seen through it. The anterior larval respiratory cornua are very small and ochraceous. The posterior spiracular tubes forming the posterior respiratory appendage are seen slightly raised above the surface of the last segment in the body which is entirely greenish yellow. They are of a light brown colour. At the end of the respiratory appendage on each side are three digitate spiracles, four small inter-spiracular spurs and a circular dorsal plate as shown in Plate LXIII, fig. *d*.

The mouth parts of the larva consist of inverted V-shaped jaws and three pairs of mouth hooklets. The outer pair of mouth hooklets are small and tooth-like, the other two pairs, one at the anterior and the other at the posterior end of the jaws, are elongated and rod-like.

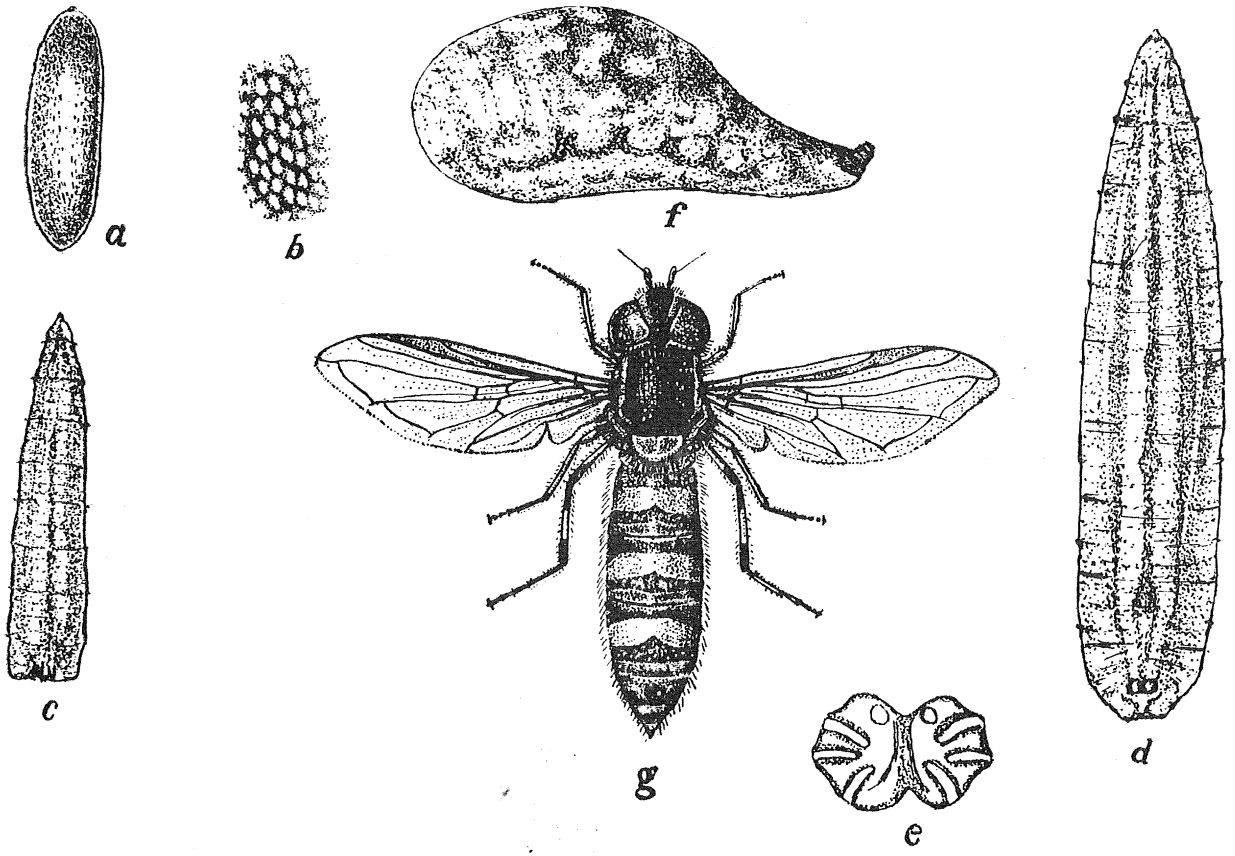
The larval period in this species was found to be about a week in May, 1931.

Pupa (Plate LXI, fig. *c*)—The pupa is 6·1 mm. long and 2·3 mm. broad. It is of a pale ochraceous colour. The ventral surface is flat and the dorsal, just as in other species, humped. The body of the pupa is broad and rounded anteriorly, narrowing gradually towards the posterior end on which is found the posterior respiratory appendage. The skin is wrinkled as in the larva. The inter-segmental spines are very prominent. The pupal period is about one week. Towards the end of this period, the pupa becomes dark, specially more in the anterior half of its body. This is due to the colour of the fly within the pupa.



Paragus serratus Fabricius.

(*For explanation please see p. 570*).



Sphaeroporiajavana Wiedemann.

(*For explanation please see p. 570*).

LIFE-HISTORY OF *Sphaerophoria javana* (WIEDEMANN)

Introduction

While making observations in the field and working in the laboratory on the various stages in the life-cycle of *Baccha pulchrifrons* Aust., it was found that there were also larvae of another species of Syrphid fly preying upon nymphs of *Ctenophalara elongata*. These larvae were green in colour in the adult condition, with a pair of fairly broad median dorsal fat stripes. The flies bred from these larvae were identified as *Sphaerophoria javana*. It may be stated that the fly has never so far been recorded from Bihar. Collections of this species have been made from Coorg, South India, 15-20. X. 1915 (Fletcher); above Tura, Assam (*Kemp*). It has also been recorded from Ceylon, Sumatra and New Guinea. Fletcher reared a fly from larva which he found feeding on Psyllids on a tree in Shillong in the beginning of November, 1918. It was in the months of November and December, 1931, that the eggs and larvae of this species were collected at Pusa and flies reared. Later in the month of January, 1932, the larvae of this species were found feeding on aphids on cotton plants and a male was reared.

Egg (Plate LXII, fig. *a*)—Length ·89 mm., breadth ·29 mm. Freshly laid eggs are white, comparatively narrower than those of *Baccha pulchrifrons* which are also found on the same leaf of *Bombax malabaricum* in association with the nymphs of the Psyllid *Ctenophalara elongata*. The chorion of the egg is marked with elongated oval areas all over the surface without any processes on the sides. The egg assumes a darker hue with a purplish tinge at the time of hatching. The incubation period is about 48 hours.

Young larva (Plate LXII, fig. *c*)—The newly hatched larva is 2·33 mm. long and ·48 mm. broad. It is pale yellow in colour with a slight greenish tinge, equally broad in the posterior half and gradually tapering towards the anterior end. At a little later stage the larva becomes more yellow, the middle portion being bottle-green, and a reddish tinge appears in the fourth and fifth segments of its body. A pair of median fat lines, much more prominent in the full-grown larva but not so in the young stage, can be seen enclosing between them the median dorsal blood vessel which is reddish in colour. The anterior larval respiratory cornua are distinctly seen as two small light ochraceous processes, one on each side between the head and the prothoracic segment. The posterior larval respiratory tubes, ochraceous in colour, are quite separate and are situated on the last segment. The inter-segmental spines are small and of pale ochraceous colour.

Full-grown larva (Plate LXII, fig. *d*)—Length 10 mm., breadth 2·0 mm. The full-grown larva is pea-green in colour. Mid-dorsally there are two fairly broad fat stripes extending from the ninth to the fourth segment. The stripes appear green

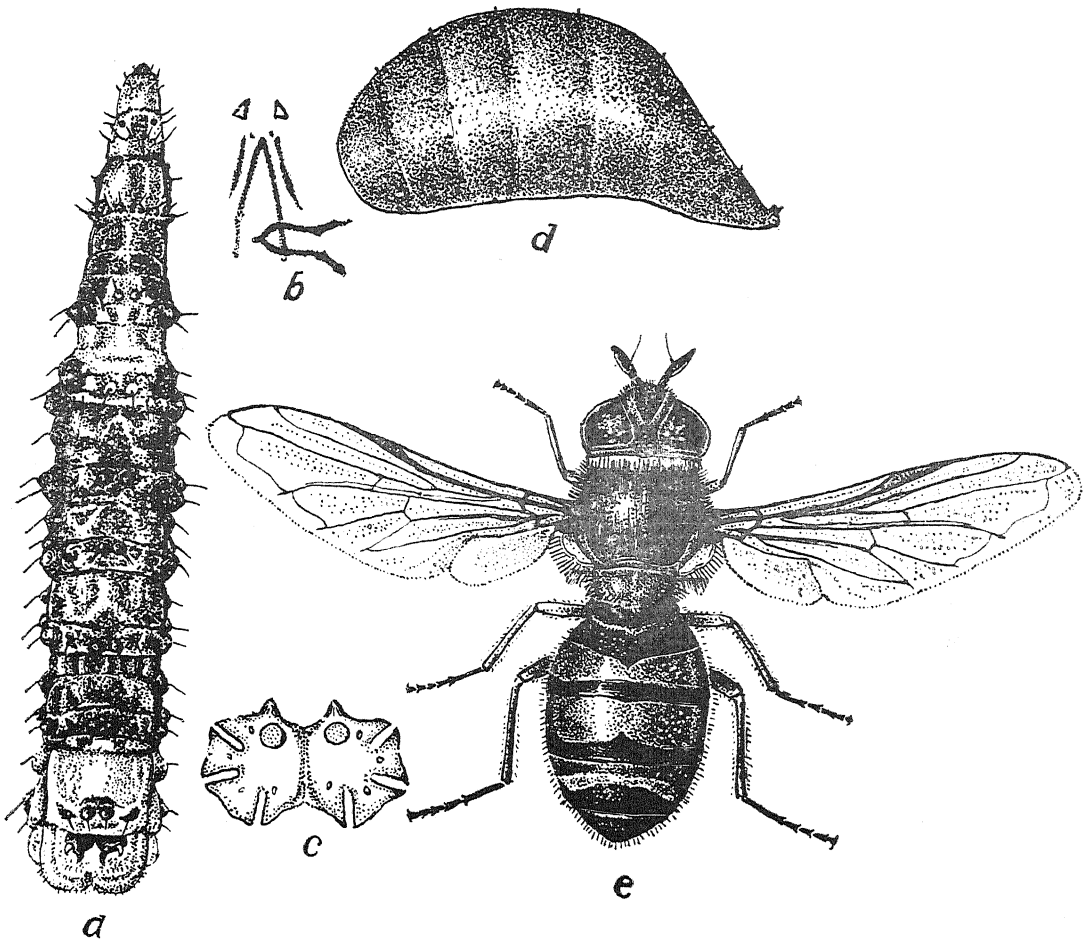
due to the general body colour of the larva being green. The body of the larva is uniformly broad posteriorly and is seen to narrow very gradually towards the anterior end. The head of the larva is small, conical, being composed of two segments. It encloses within it the œsophageal frame-work containing the jaws and the mouthhooklets. Each arm of the inverted V-shaped upper jaw possesses three teeth which, when the jaws are in action, serve the purpose of puncturing the body of the victim at various places. Both upper and lower jaws are heavily chitinized. The outer mouthhooklet of each side is bidentate and is of a black colour. There is only a single pair of lateral mouthhooklets. They are situated one on each side of the jaws and are much less chitinized than the other mouth parts. They are long and slender, broad anteriorly, gradually narrowed towards the posterior end.

After the head, ten clear segments can be counted. The inter-segmental spines are small, ochraceous and can only be seen in the adult larva by the help of a microscope. The skin of the larva is thrown into folds or wrinkles and three such wrinkles can be counted in most of the segments. The entire course of the tracheal tubes can be seen beneath the green translucent skin of the body of the larva. Mesially on the dorsal surface of the last segment can be seen the posterior respiratory appendage, composed originally of two chitinous posterior respiratory tubes, separate in the young larva, and closely applied by their inner sides when it is about full-grown. The respiratory appendage is of brown colour and, unlike the same appendage of the larva of *Baccha pulchrifrons*, is slightly raised above the surface of the body. In the end view of the posterior respiratory appendage (Plate LXII, fig. *e*) can be seen, on each side, three digitate spiracles and a dorsal plate. The anterior spiracles, two in number, are placed on sides at the anterior portion of the prothoracic segment at the tips of the larval respiratory cornua which are of a pale ochraceous colour. The dorsal blood vessel in the larva is of a reddish orange to purple colour. In the posterior segments of the larva it is darker. The various chambers of the vessel can be seen undergoing rhythmic contraction and expansion at the time when the larva is quiescent after a feed.

The larval period, as calculated from the data compiled, while rearing of the flies was carried in the laboratory, varies from 10-15 days.

Pupa (Plate LXII, fig. *f*)—Length 5·1 mm., breadth 2·1 mm. The pupa is green in colour. Like the pupae of other aphidophagous species of Syrphidae it possesses a flat ventral surface and is inflated dorsally. It is fusiform in outline, being broad and rounded anteriorly and gradually tapering towards the posterior end which bears the posterior respiratory appendage. In a newly formed pupa, the two fat stripes, the inter-segmental spines and the wrinkles on the skin of the body segments can be seen as in the larva. The posterior respiratory appendage is

PLATE LXIII.



Syrphus serarius Wiedemann.

(*For explanation please see p. 570.*)

shining brown in the beginning but later on becomes darker. The ventral surface is lighter in colour than the dorsal surface.

While breeding flies of *Sphærophoria javana* two different types of colouration were noticed in the pupae. In one type the colour of the pupa is as described above and from it male fly was seen to emerge. In the second type the pupa is equally green on the dorsal as well as the ventral surface. Dorsally black patches can be seen over the green surface presenting the appearance of five pairs of laterally placed oblique stripes. The ventral side in this type of the pupa developes small black dots on the sides. From this type of pupa female fly was seen to emerge. Towards the end of the pupal period which is of eight to ten days' duration, the colour of the eyes, thorax and abdomen of the imago can be seen through the pupal skin, the anterior third of which appears reddish-brown, the middle portion black and the hind portion black with greenish yellow stripes.

Imago (Plate LXII, fig. *g*)—A sufficient number of flies, both males and females, were reared in the laboratory from the eggs, and larvae collected in the field. In fresh specimens thus obtained, the colour of the face, thorax and abdomen was lemon-yellow instead of orange-yellow as found in the description of the species given in the Fauna of British India, Diptera, Vol. III, pp. 100—101. In all other respects the specimens exactly tallied with the named ones in the Pusa collection and answered perfectly to the description of the male and female given in the Fauna Volume.

Life-cycle

The life-cycle of *Sphærophoria javana* takes about three weeks to complete. The egg hatches in about forty-eight hours after it is laid. The larva, under proper laboratory conditions, pupates 10 to 12 days after the hatching takes place and the adult fly emerges 8 to 10 days after pupation of the larva.

LIFE-HISTORY OF *Syrphus serarius* WIEDEMANN

Syrphus serarius Wied., is a hill species and has been recorded from various hill stations, such as, Masuri, Kumaon, Almora, Murree, Darjeeling, Shillong and other places besides Pusa, Coorg (S. India) and Ceylon. It has been reported from Java, and Coquillett records it from Japan.

The larvae of this species were found feeding on aphids on mustard in Pusa in February and March 1932. The eggs could not be obtained and hence the description of only the larva and pupa is given below.

Full-grown larva (Plate LXIII, fig. *a*)—The adult larva is 9·1 mm. long and 1·5 mm. broad. In the earlier stages the larva is black both ventrally and on the

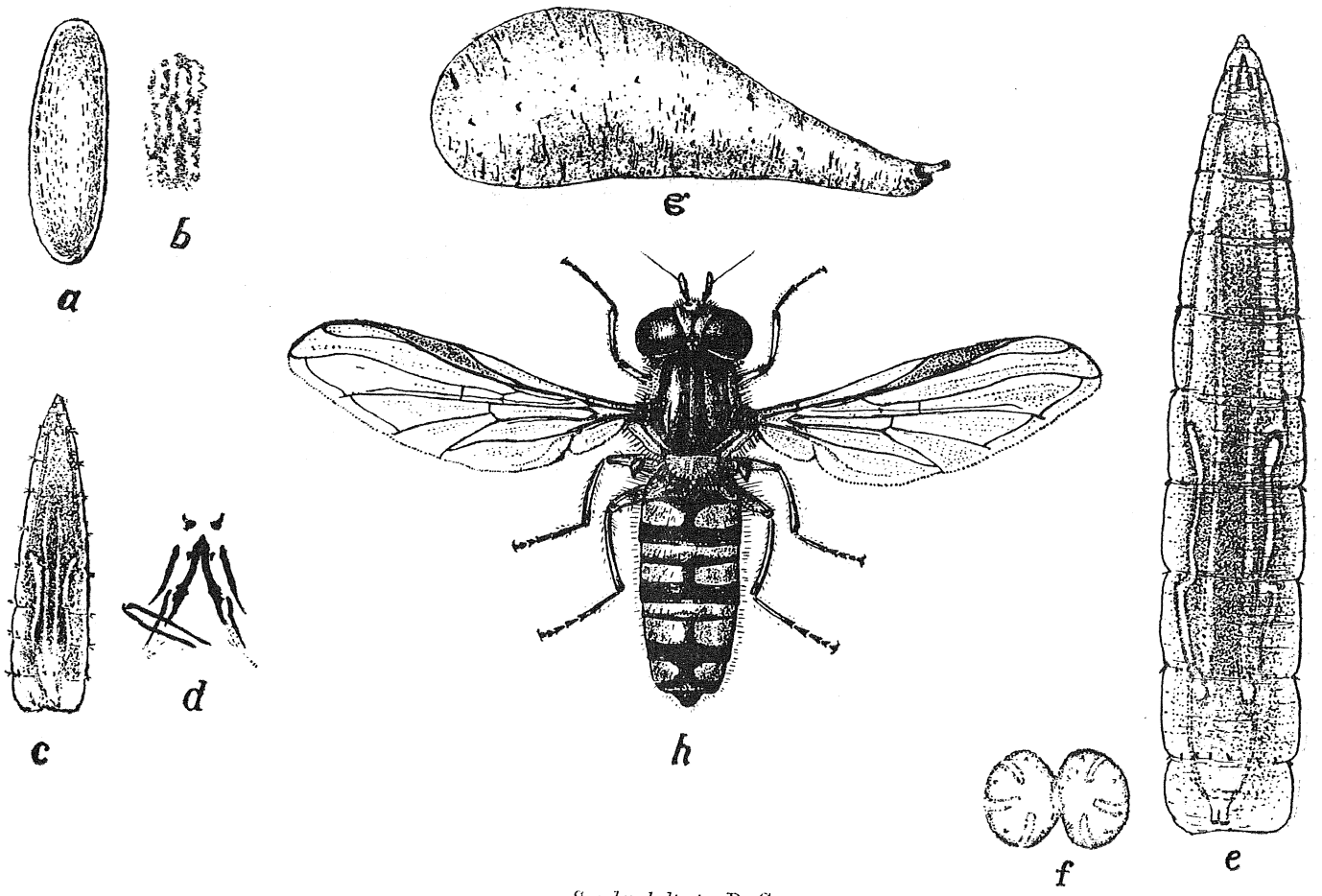
dorsal surface excepting the 4th, 11th and the 12th segments which are white above due to the development of fat. The larva, like those of other species of aphidophagous Syrphidae, is broad posteriorly and is gradually seen to narrow towards the anterior end. The skin is tough. The body is covered all over with small black bristly hairs which can even be seen on the 4th, 11th and the 12th segments which are white. The tracheae can not be seen through the tough skin due to its black colour. The anterior larval respiratory cornua are pale in colour and can be seen on the anterior side of the prothorocic segment, one on each side. The posterior larval respiratory tubes are seen joined by their bases. They are shining black and are slightly raised above the surface of the segment on which they are situated. At the end of each tube will be found three digitate spiracles, four inter-spiracular spurs and a prominent shining dorsal spine. The wrinkles on the segments are as in other larvae of the aphidophagous species. The inter-segmental spines are black and very prominent. The median spines are situated a little in front of the dorsal and dorso-lateral spines. There are also two rows of small spines in the two head segments. The bases of the median spines are sometimes white in some of the segments in the body.

The fat in the larva increases as it approaches maturity. The colour of the larva, when it is about to pupate, is as follows: Ventral side of the larva black; dorsal surface black, excepting a patch of white on each side of the head and the first segment; sixth and the anterior portion of the seventh segment white; eighth, ninth and tenth segments partially white due to irregularly distributed white fat patches; eleventh segment entirely white and twelfth segment white excepting the posterior respiratory tubes which are shining black.

The mouth parts of the larva are a pair of inverted V-shaped jaws and three pairs of mouth hooklets. The outer pair of hooklets are tooth-like, each possessing a broad base and pointed apex. Out of the two pairs of lateral mouth hooklets on the sides of the jaws, there is a pair of rod-shaped mouth hooklets. The second pair of mouth hooklets are very small chitinous structures on the sides of the jaws at their anterior end.

The adult larva has a peculiar habit of bending its body at times while it is feeding. Sometimes in this position it is easily mistaken for a caterpillar.

Pupa (Plate LXIII, fig. *d*)—The larva pupates either on the leaf or on the fruit of mustard. The newly formed pupa is fusiform, broad and rounded anteriorly and narrowing towards the posterior end on which is situated the posterior respiratory appendage. It is 6·4 mm. in length, 2·8 mm. in height and 3·1 mm. in breadth. The inter-segmental spines are not so conspicuous as in the larva. The wrinkles on the segments are as in the larva. The pupa is black ventrally, and on the dorsal surface besides the black colour white patches of larval fat can be seen just as in the



Syrphus balteatus De Geer.

(*For explanation please see p. 570.*)

larva. After two or three days the colour of the pupa becomes light ochraceous, the anterior portion being dark, and also there is a broad diamond-shaped area of a dark colour about the middle of the dorsal surface. The pupal period is about eight days.

LIFE-HISTORY OF *Syrphus balteatus* (DE GEER)

Introduction

The fly *Syrphus balteatus* (De Geer) is one of the commonest species of the genus *Syrphus* and has been reported from all over India. In Pusa the fly is fairly common and is available during most of the months in the year. From January to March the flies of this species are seen in a sufficient number in the fields and can be collected at any time in the day. They are seen hovering over flowers in search of food which they find in honey stored in the nectaries of the flowers. The gravid female besides looking for the honey will be seen here on this leaf, there on another and ultimately will be out of sight and if these leaves are examined with a field lens carefully, one may find on any one of them on its underside a newly laid egg in a small colony of green aphids.

The adult flies of this species can be recognised at a glance in the field by the orange coloured face, shining aeneous thorax and orange coloured abdomen with black segmentation, and, in most of the specimens, with an additional narrow black stripe in each one of the third and the fourth segment.

Field collections of the eggs and the larvae of this species were made on a large scale in a cotton field in January and February, 1932. The larvae were found both on the underside of the leaves preying upon nymphs of the cotton aphis and on the thalami of flowers which had just opened, where they were found mainly feeding on young nymphs of Coccids which attack the shoots of cotton plant. It is in the latter position that maggots of the Drosophillid, *Getonides perspicax* Knab, are also found but it is not difficult to distinguish the larvae of one from the other. The young, and in some cases the adult, larva of *Syrphus balteatus* (De Geer), is black on the posterior two thirds of its body, the anterior third being light yellowish grey. In the posterior portion of the body of the larva will be found a red coloured loop underneath the skin.

All the stages in the life-cycle of this common Syrphid fly were carefully studied in the laboratory and the life-history was thus worked out.

Egg (Plate LXIV, fig. *a*)—The eggs are 1·1 mm. in length and ·35 mm. in breadth. They are singly laid on the under-surface of the cotton leaf. Freshly laid eggs are milk-white, elongated oval in outline and rounded at both the ends. The chorion of the egg, when examined under the microscope, is seen ornamented

with elongated chalky areas arranged in longitudinal rows parallel to the long axis of the egg. On both the sides of each such area are seen small dentate processes. The egg, when about to hatch, becomes dirty white. The incubation period is about 48 hours.

Young larva (Plate LXIV, fig. *c*)—The young larva is 1·4 mm. long and ·35 mm. broad. The newly hatched larva is of black colour dorsally. It is broad posteriorly and gradually narrows towards the head segments. The flat ventral surface of the larva is pale grey, so also are the sides, the head, the prothoracic segment and the last body segment bearing the chitinous posterior respiratory tubes. A pair of thin white fat streaks can be seen extending from the posterior to the anterior end on the median dorsal surface of the body of the larva. The dorsal blood vessel is not clear owing to the black colour of the larva. A pair of red tubular markings on the posterior surface of the larva gives a characteristic appearance to it by which it can be easily recognised in the field. The rows of small inter-segmental spines can be seen with a hand lens, the first between the head and the prothorax and the last between the 11th and the 12th segments. The tracheal tubes can be seen within the skin throughout their entire course in the body of the larva. The pale anterior larval respiratory cornua are very small but can distinctly be seen. The posterior chitinous spiracular tubes on the last body segment are separate.

Full-grown larva (Plate LXIV, fig. *e*)—The adult larva is 9·1 mm. long and 1·6 mm. broad. There are in all twelve segments in the body of the larva. On the last segments is seen the ochraceous posterior respiratory appendage which is about ·4 mm. above the surface of the segment. At its end (Plate LXII, fig. *f*) on each side are three digitate spiracles, a circular plate and four interspiracular spines. The colour of the body is pale ventrally, black on the dorsal surface from the ninth to the fourth segment. The anterior four segments are pale grey. The red tubular n arking on each side of the body extending from the sixth to the eighth segment stands out in contrast to the black colour of the segments over which it extends. The tubular area of each side encloses fat within and around it on the sides. The dorsal blood vessel cannot be made out due to the black colour of the larva. The inter-segmental spines are microscopic.

The mouth parts (Plate LXIV, fig. *d*)—The mouth parts consist of a pair of inverted V-shaped jaws and four pairs of mouthhooklets. Near the distal end of each limb of the upper jaw can be seen three small teeth. The outer mouth hooklets are conical with sharp apices. The other three pairs are lateral, a pair of very small comma-shaped mouth hooklets and two pairs of rod-like mouth hooklets.

The black colour dorsally on the body of the larva is not constant and has been found to vary to a great extent in the larvae both obtained from the field and

reared in the laboratory. Some larvae of this species collected in the field on cotton aphis in December, 1931 and January, 1932 and others on cabbage aphis about the first week of March, 1932, were white, each with a red tubular marking on its sides. Larvae reared in the laboratory from eggs of this species collected in the field also developed black colour to a varying extent. The black colour disappears in the larva as it is about to pupate. At this stage the larva presents a white appearance, the colourless dorsal blood vessel is seen surrounded by fat on its sides, there being more fat within the tubular marking and around it on the sides. The Table given below shows the larval period in *Syrphus balteatus* (De Geer).

TABLE IV

Showing the larval period in Syrphus balteatus (*De Geer*)

Larva No.	Date of hatching of the larva	Date of pupation of the larva	Larval period
			Days
1	17th December 1931 . .	28th December 1931 . .	11
2	18th December 1931 . .	28th December 1931 . .	10
3	23rd December 1931 . .	5th January 1932 . . .	13
4	24th December 1931 . .	6th January 1932 . . .	13
5	26th December 1931 . .	9th January 1932 . . .	14

Pupa (Plate LXIV, fig. *g*)—The pupa is 5·9 mm. in length, 2·1 mm. in breadth and 2 mm. in height. It is flat ventrally, broadly rounded anteriorly and is seen to narrow gradually towards the posterior end on which is situated the respiratory appendage. In colour it is light ochraceous. The inter-segmental spines are microscopic. In some cases there are small black dots on the dorsal surface and the sides of the pupa, the dots being more closely situated on the sides than on the dorsal surface. Besides the dots, there are in this type of pupa, three to four narrow black stripes in the rounded anterior region, and the same number of small black areas mid-dorsally. The respiratory appendage in all the pupae examined is of a pale ochraceous colour.

As a sufficient number of flies were reared from both kinds of pupae, it appears that difference in the markings on the surface of the pupa has nothing to do with the sex of the imago. This difference in the marking of the pupa in one and the same species was also noticed in *Baccha pulchrifrons* and *Sphærophoria javana*, and in the latter, the difference in marking had a bearing on the sex of the flies which emerged from them.

At the time when the fly is about to emerge, the anterior rounded portion of the pupa becomes red, the middle portion black and the posterior black with orange stripes. These colours correspond to the colour of the head, thorax and abdomen of the imago.

The pupal period varies from 9 to 11 days.

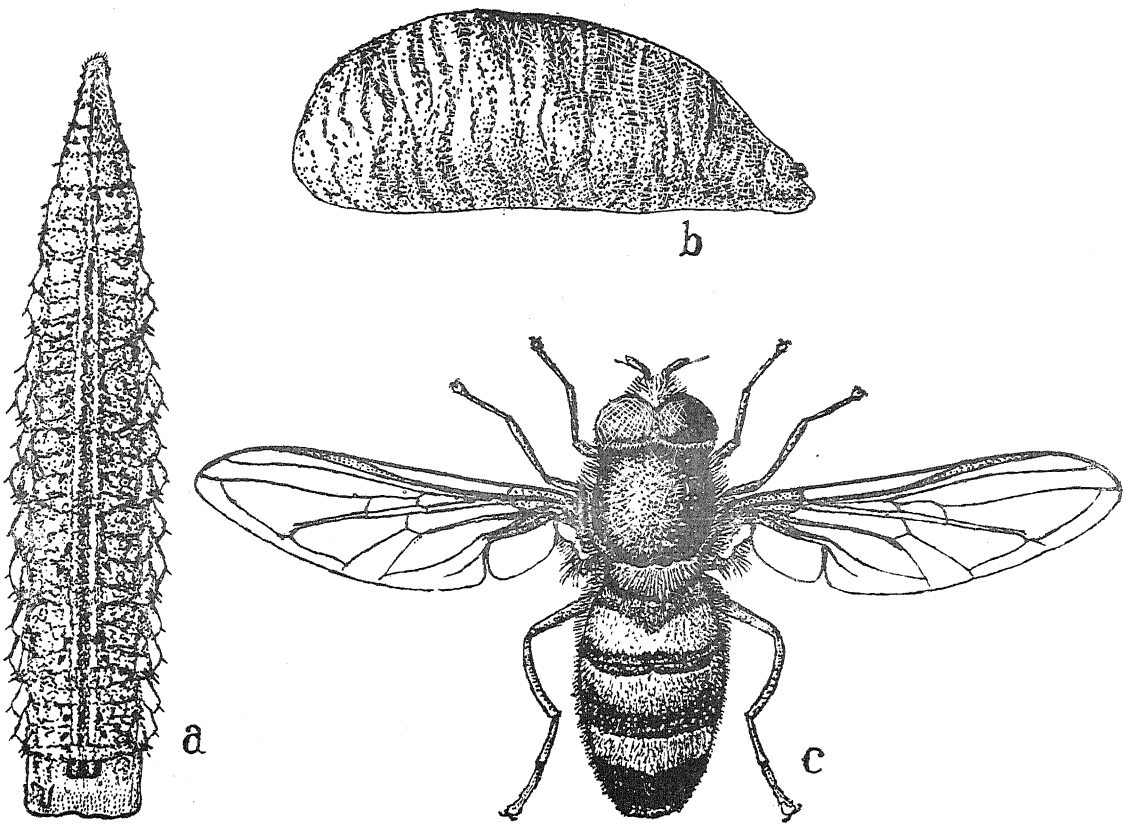
Life-cycle

The whole life-cycle occupies about three weeks. The egg hatches in about 48 hours after it is laid. The larva feeds for about 10 days after which it pupates. The fly emerges 10 days after the pupation of the larva.

Imago (Plate LXIV, fig. *h*)—A number of flies, both male and female, were reared in the laboratory. It was found that the orange colour in the second, third and fourth segments of the abdomen varied in different specimens. Brunetti [1923] in his Fauna Volume on Syrphidae describes the abdomen as " almost linear, slightly broader about the end of the second segment, wholly orange, varying a little in shade, and indistinct black median spot on the first segment united to a black stripe on the hind margin; this does not reach the sides, but is continued on second segment as a transverse basal band joined by a median stripe of varying width to a broad black band on hind margin, this latter band uniformly wide and reaching sides; third segment with a narrow transverse line of uniform width (in Indian specimens), or narrowed to a point on each side and also interrupted in the middle (in European specimens), seldom reaching sides; hind margin with a black band as on the second segment; fourth segment similarly marked except that the hind marginal band leaves the extreme margin pale and a slightly convex anteriorly or straight in some cases and practically or actually terminal; fifth segment orange with an indistinct small black spot above the middle ".

In some specimens bred in the laboratory, the orange and black markings on the abdominal segments are just as described above but in others (Plate LXIV, fig. *h*), it differs from the above in following respects:—The second, third, fourth and fifth segments are black with orange spots, a pair in each segment, rectangular in the third and fourth segments and triangular in second and fifth, with their apices directed towards the middle of the segments on which they are situated, third and fourth segments with a pale yellow stripe in the anterior margin and the posterior border of the fourth segment indistinctly pale yellow. The narrow black line on the third and fourth segments of the specimens with abdomen wholly orange yellow can be seen here occupying the same position but not so marked. In all other details these specimens answer perfectly to the description of the species given in the Fauna Volume. This small difference in the markings of the abdominal

PLATE LXV.



Syrphus confrater Wiedemann.

(*For explanation please see p. 570.*)

segments is only a minor variation within the range of a species and therefore it is not necessary to create a new variety.

LIFE-HISTORY OF *Syrphus confrater* WIEDEMANN

Syrphus confrater Wied., has been reported in India from the Punjab, Bihar, Bengal, Assam and the Madras Presidency. The larva of this species has been reported as feeding on aphis on cotton, wheat, cabbage and chrysanthemum. In Pusa the fly is fairly common from January to March. The larvae of this species were found on mustard plant feeding on aphids. Mr. Fletcher in 1923 found the larvae of this species feeding on *Eriosoma lanigera* (woolly aphis) on pomegranate in Kashmir.

The eggs could not be obtained. The descriptions of larva and pupa are given below.

Full-grown larva (Plate LXV, fig. *a*)—The adult larva is 16·8 mm. long and 3·5 mm. broad. It is larger in size than any other larva of the aphidophagous species of Syrphidae reared so far in Pusa. The colour of the larva is light orange. The body is uniformly broad for the two thirds of its entire length from the posterior end in front of which it is seen to narrow towards the head. There are 12 segments in the body of the larva, the last bearing dorsally two posterior respiratory tubes each bearing at its end the spiracles by which the tracheal tube opens. The median dorsal blood vessel is darker in colour and its various chambers are seen when the larva is at rest. A pair of fat streaks are seen, one on each side of the dorsal blood vessel, and besides these the fat is distributed irregularly in the form of white spotted areas on all the segments. The dorsal surface is darker in colour than the ventral due to the presence of minute black bristly pubescence which is more pronounced on the sides of the fat streaks. The area around the fat streaks on this account appears darker. The skin is much less translucent than that of the larvae of other species and hence the tracheal tubes cannot be seen through the skin. The inter-segmental spines are quite prominent in the younger stages of the larva but are rather microscopic in the adult larva. There are, as in the larvae of other species, three wrinkles on the surface of each segment in the body excepting the head, the prothoracic and the last segment. The latter appears darker than other segments due to the more conspicuous black bristly pubescence.

Pupa (Plate LXV, fig. *b*)—The pupa is 8·75 mm. long, 3·5 mm. broad and 3·3 mm. in height. It is of a dark brown colour, the ventral surface being lighter in colour than the dorsal. The body is fusiform, being broadly rounded anteriorly and gradually tapering towards the posterior end bearing the small shining black respira-

tory tubes. The inter-segmental spines are very small and pale ochraceous. The wrinkles in the segments are as in larva. The pupal period is about 12 days.

LIFE-HISTORY OF *Syrphus isaaci*, SP. NOV.

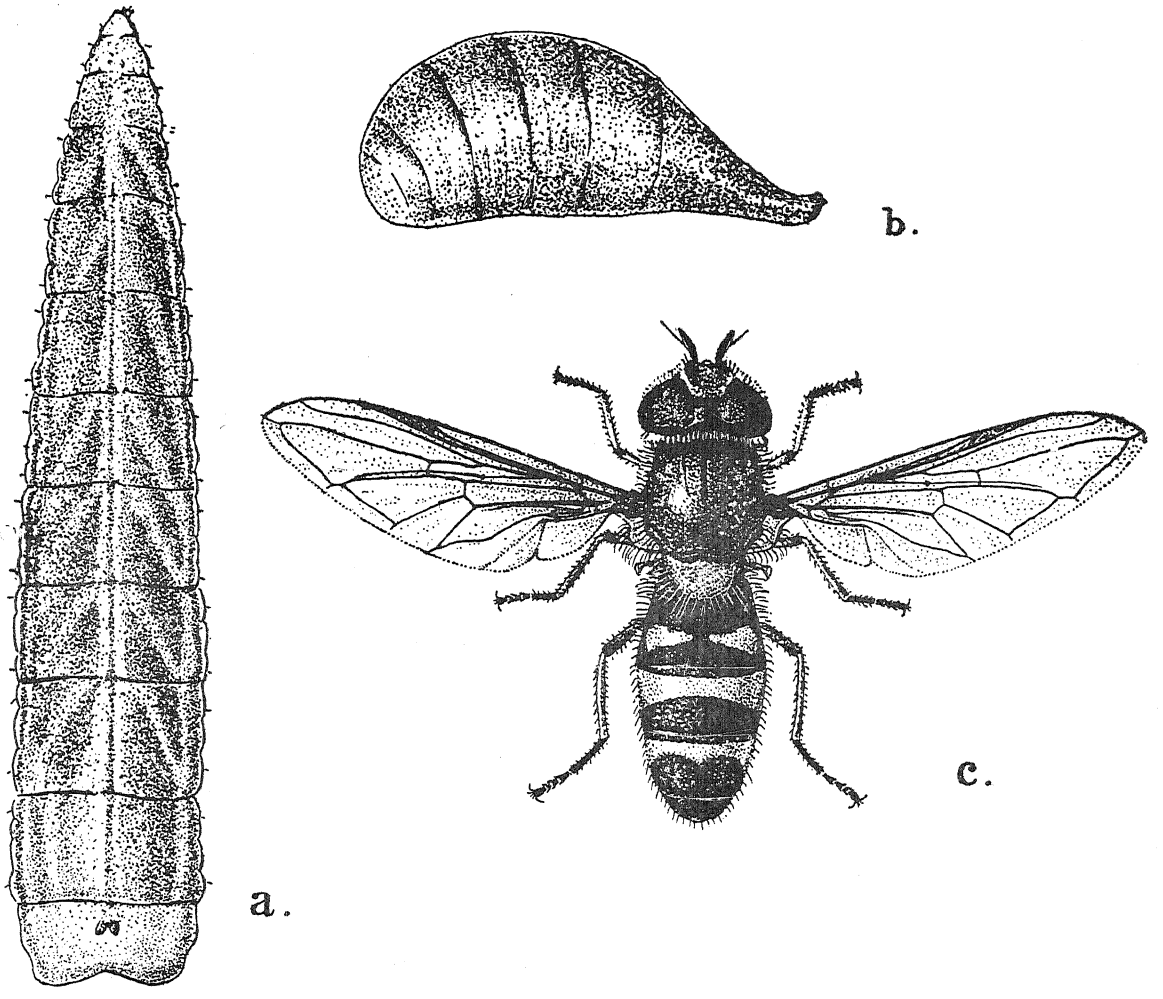
Syrphus isaaci has been recorded from Mangaldai sub-division, Assam [Kemp, 1910]; Nagarkote (Nepal); Masuri; Kumaon and Pusa. A few specimens of this species in the Indian Museum collection and a few in the Pusa collection bear the label *Syrphus instabilis* Brun. The description of *Syrphus instabilis* Brun. has not been published and therefore the senior author has taken the liberty to describe it under a new name as above.

The larvae of *Syrphus isaaci* were found feeding on mustard aphis in Pusa, in February 1932. The description of the adult fly, male and female, is given after that of the larva and the pupa.

Full-grown larva (Plate LXVI, fig. *a*)—The adult larva is 14 mm. long and about 2·6 mm. broad. It is of a light pink brown colour, flat ventrally, broad posteriorly and gradually narrowing towards the anterior end. The segments in the body of the larva are marked by inter-segmental furrows which are three in number in each segment excepting the last in which they are absent, and third and fourth segments in which there are one and two furrows respectively. The body is covered dorsally by light brown bristly pubescence hardly visible to the naked eye. The inter-segmental spines are small and ochraceous. The dorsal blood vessel is slightly darker and is surrounded on both sides by fat forming the median dorsal fat streak. Inter-segmentally, from the median fat streak, obliquely placed light coloured fat stripes are seen to arise and meet on each side into a long white fat stripe as shown in the diagram of the larva. This arrangement of fat stripes gives a characteristic appearance to the larva. The tracheal tubes cannot be seen through the tough skin of the larva. The posterior respiratory tubes are brown in colour. They are slightly raised above the dorsal surface of the last segment on which they are situated.

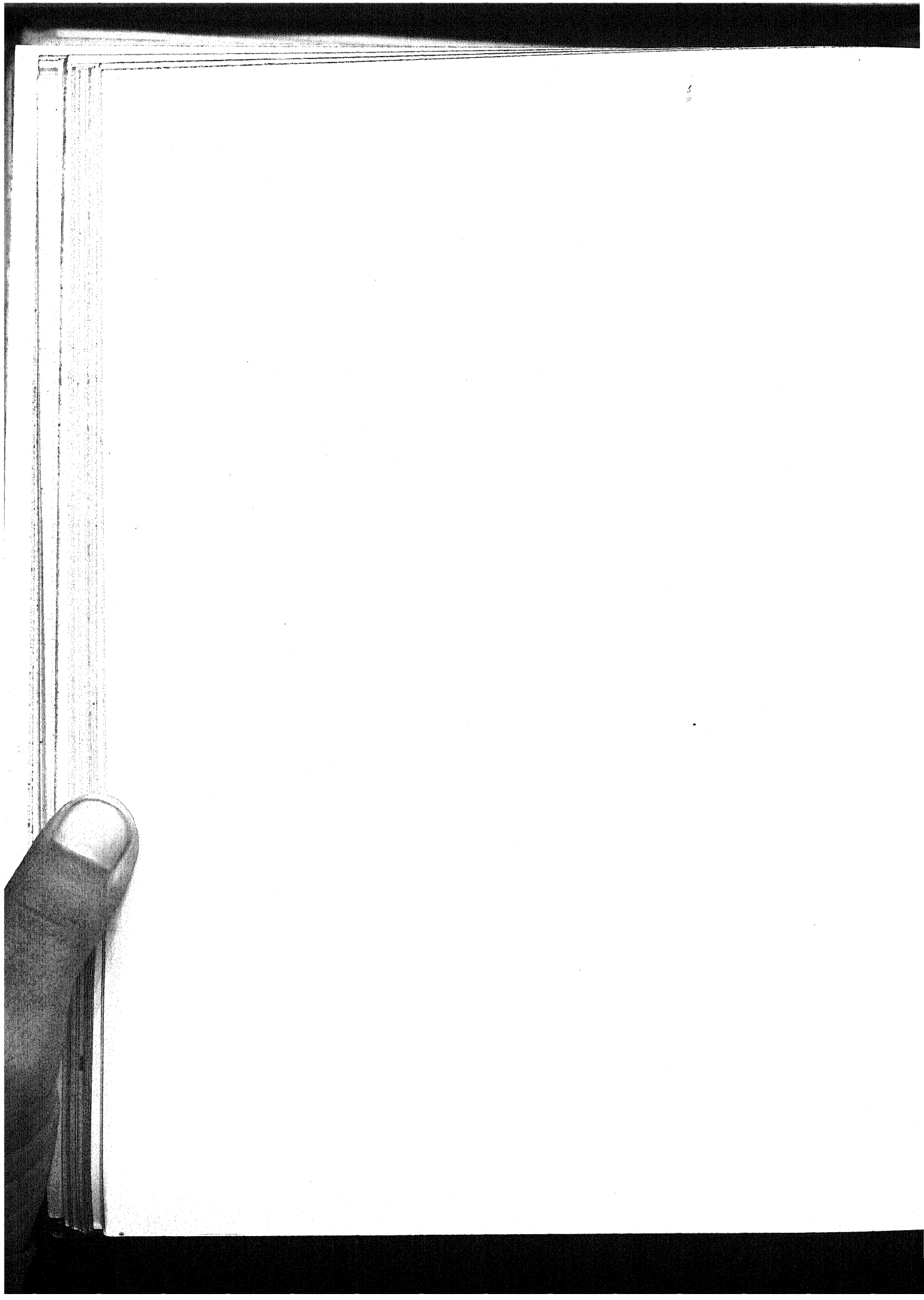
Pupa (Plate LXVI, fig. *b*)—The pupa is 7·0 mm. long, about 2·7 mm. broad and 3 mm. in height. In shape it is broadly rounded anteriorly where it is high and is seen to narrow gradually towards its posterior end. The ventral surface of the pupa is flat. The dorsal blood vessel is seen as a dark line mid-dorsally. The inter-segmental spines are ochraceous in colour and microscopic. The segmental furrows are as in the larva. The posterior respiratory tubes are small bearing a metallic lusture. The pupal period is about two weeks in February 1932.

Adult fly, male and female (Plate LXVI, fig. *c*)—Male: head—eyes brown with dark brown pubescence, whitish at the lower angles; vertical triangle small, black



Syrphus isaaci sp. nov.

(*For explanation please see p. 570.*)



with black hairs; upper part of the frons yellow with black hairs. Antennal prominence shining black, space around the base of antennae very narrowly orange; face light yellow with white pubescence all over up to the oral margin, the latter with short white hairs on the sides, the central hump dark brown, bare; cheeks very narrow, cinereous with white pubescence. Fringe of hairs on the posterior margin of the eyes orange yellow. Female: vertex shining violet, eyes separated, the space between the eyes greatest at the level of the antennae, narrowing gradually towards the vertex.

Thorax moderately shining black with a slight æneous tinge, with conspicuous yellow pubescence on the sides and dorsum; pleurae, cinereous with light yellow fine hairs. Scutellum translucent, dull yellow, with long black hairs on its dorsal side and yellow hairs fringing the margin.

Abdomen black; first segment shining æneous with a bluish tinge; second segment with a pair of whitish (in the 'Type' specimen) to light orange narrow triangular spots, their apices separated, their bases nearly reaching the side margins of the segment; third segment with a narrow transverse band of orange colour near the anterior margin, the band a little broader near the sides than in the middle; fourth segment anteriorly with a similar band, lighter and narrower than the band in the third segment; posterior portion of the fourth and fifth segments somewhat shining. Hairs on sides of the abdomen light yellow mixed with black at places, prominent on the sides of the segment. Dorsally the pubescence black on black parts and yellow on orange parts with few yellow hairs on the black areas. Venter blackish, slightly metallic and with bands corresponding to those on the dorsal side.

Legs yellowish brown; nearly the basal half of the anterior femora, outer sides of the middle femora, the entire hind femora, an indistinct ring at the middle of the hind tibia, also all tarsi slightly darker. Wings clear, stigma pale yellow; halteres light orange. Lengths: 10 mm., of wing 8·6 mm.

Type male in Indian museum. Type female in Pusa Collection.

LIFE-HISTORY OF *Helophilus bengalensis* (WIEDEMANN)

Helophilus bengalensis (Wied.) is common in Bihar and Bengal. The species is apparently widely distributed throughout India as it has been reported from Katmandu (Nepal), Calcutta, Pusa, Bangalore and Sibsagar.

The flies of this species are available in Pusa during the rainy season. They have been collected in the months of July, August and September. They were observed during these months in the fields hovering over wild plants near the river bank in Pusa. Egg laying was never observed in the field. Males and gravid females, collected in the field in September were brought to the laboratory and liberated together in cages, each consisting of a glass bell jar within which was

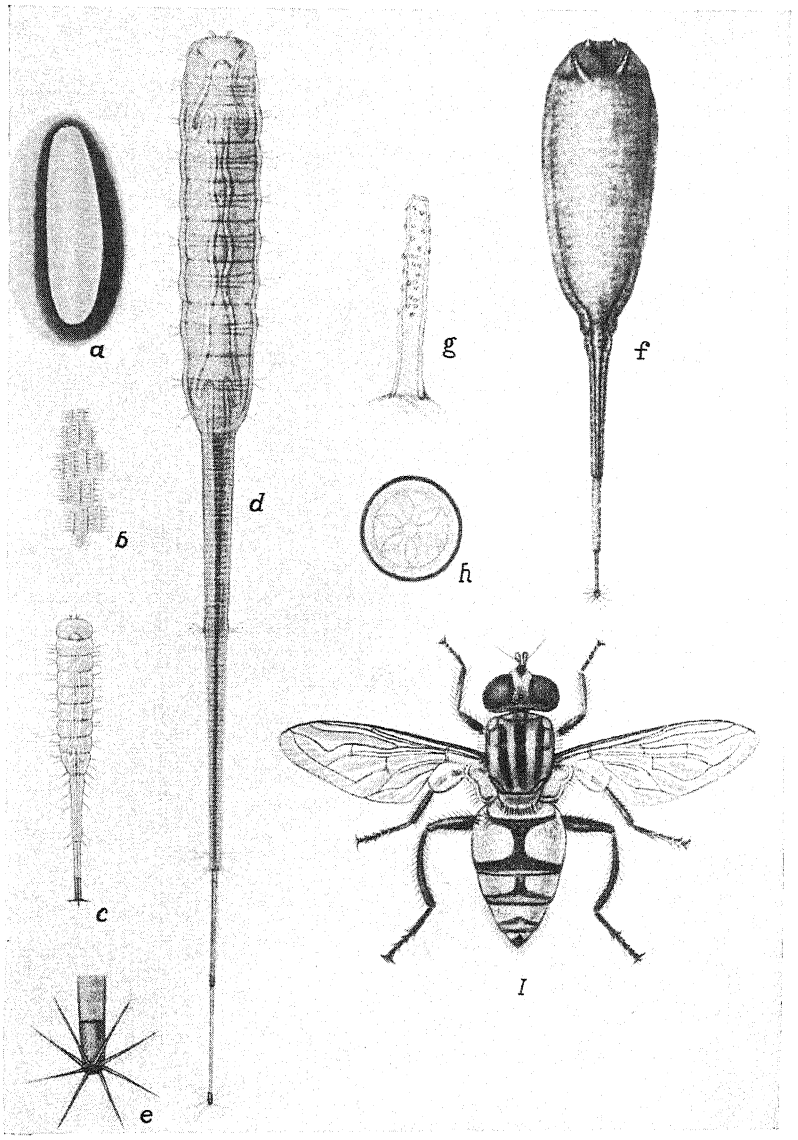
placed a small Petrie—dish containing a piece of cotton soaked in sugar solution and a small leafy branch of a wild plant immersed in a beaker half full of water. Next day, or two days after confinement of the flies in a cage, small masses of white eggs (in some cases a single big mass of white eggs was observed) were observed deposited on the sides of the cage, on a leaf of the branch, or even in the Petrie-dish containing the piece of cotton soaked in sugar solution.

Egg (Plate LXVII, fig. *a*)—Freshly laid eggs are white with a creamy tinge about their middle, each measuring 1·0 mm. in length and ·30 mm. in breadth. The egg is elongated oval in outline, rounded at both ends, the anterior bearing the small micropyle being slightly narrower than the posterior end. The ventral surface of the egg is flat and the dorsal a little convex. The chorion is marked all over with elongated rod-shaped areas in longitudinal parallel rows, each such area giving small branches on both the sides. These small branches do not meet one another.

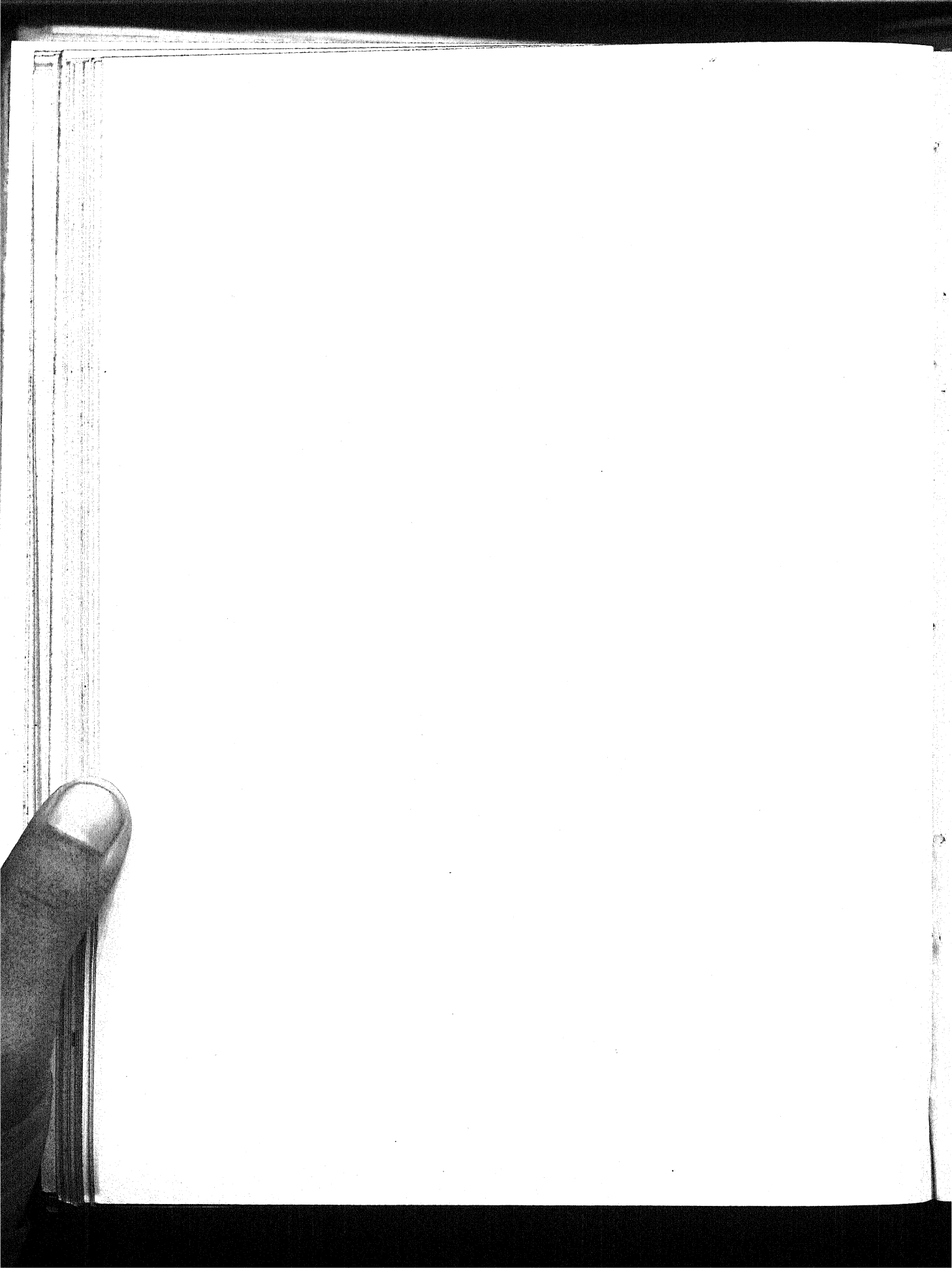
Food of the larva—The larvae were fed on decaying wood in the act of fermentation and freshly killed fly maggots. They were observed to get into the body of the crushed maggots, thereby devouring entirely the inside viscera. They throve well in this medium and became full-grown in 16 days after their hatching.

Young larva (Plate LXVII, fig. *c*)—The young larva is white in colour, 5·5 mm. long and ·68 mm. broad. It conforms to the type of rat-tailed larva to which also belongs the larva of *Eristalis*. Even in the young larva, the tail, which is composed of three apparent segments, is equal in length to the body. The first segment of the tail which is a continuation of the last body segment is as long as its other two segments. The last segment of the tail is darker, bearing at its end the posterior stigmata which are ornamented with eight barbed bristly hairs as shown in Plate LXVII, fig. *e*). The two tracheal tubes are seen through the body from its anterior end to the tip of the tail. They open anteriorly by spiracles placed at the ends of the two anterior larval respiratory cornua which are not clearly visible in the young larva and at the tail end by the posterior stigmata. Ventrally the body of the larva is provided with 6 pro-legs bearing black recurved spines. The oral aperture is situated on the ventral side of the head of the larva. The anal aperture is seen ventrally on the last body segments guarded by a number of radiating digitate processes which are supposed to be renal in function.

Full-grown larva (Plate LXVII, fig. *d*)—The adult larva is 3·5 mm. in length (body and tail), and 2·7 mm. in breadth. It is pale yellow in colour. The tail is nearly twice the length of the body. The body of the larva including the tail is composed of ten segments besides the head. The tail is composed of two segments, its first apparent segment being only a continuation of the last body segment and hence cannot be taken as a separate segment. The segments in the body are marked dorsally by wrinkles in the skin. The head is lined on the margin



(For explanation please see page 570.)



and the dorsal surface by sensory spines. The two lips on the ventro-lateral aspect of the head are provided with pale hairs and weak spines. Ventrally the segments of the body are provided with 6 pairs of pro-legs as in other species of the genus *Helophilus*. The anterior larval respiratory cornua, by which the tracheal tubes open anteriorly at the junction of the first segment in the body, *i.e.*, the prothoracic segment and the head are well seen in the adult larva. They are of an orange yellow colour. The tracheal tubes are light greyish yellow and present a zigzag appearance in the full-grown larva. Throughout their course they are seen to give numerous branches to the skin, the alimentary canal, the salivary glands and anteriorly to the head and the œsophagial frame-work.

It is rather difficult to distinguish between the adult larva of *Helophilus bengalensis* (Wied.) and that of *Eristalis quinquestriatus* (Fabr.) which has also been bred at Pusa. They are very similar in general appearance, size, colour and many other details of external anatomy. The task of distinguishing between the two larvae will not be found difficult if the last segment in the tail of the larvae is examined. In the larva of *Helophilus bengalensis* (Wied.) it is pale yellow with the tip black, while in the larva of *Eristalis quenquestriatus*, it is uniformly pale yellow.

Pupa (Plate LXVII, fig. *f*)—The larva under laboratory conditions feeds for 16 days after which it pupates. For pupation it prefers moist saw-dust.

The pupa is 18·5 mm. in length (body 9 mm., tail 8·7 mm.) and 4 mm. in breadth. It is of a light brown colour dorsally, and pale yellow on the ventral side. The dorsal surface is convex sloping abruptly at the anterior end. The ventral side is flat with paired patches of small spines indicating the retracted pro-legs. Dorsally are seen two small yellow larval respiratory cornua and a little behind them the two pupal respiratory cornua which are of a pale ochraceous colour. Each pupal respiratory cornu is 1 mm. in length. The upper half of its body is studded with small spiracles appearing as dot-like tuberculated structures. Each such spiracle is shown in Plate LXVII, fig. *h*. The pupal period is about 8 days.

REFERENCES.

Barbar, H. S. (1913). *Proc. Ent. Soc. Wash.* **15**, 151-152.

Bhatia, H. L. (1931). *Ind. J. Agric. Sci.* **1**, 503-513.

Brunetti, E. (1923). Fauna of British India, Diptera, III.

Buckton, G. B. (1895). The Natural History of *Eristalis tenax* or the Drone fly.

Curran, C. H. (1920). *Canad. Ent.* **52**, 53-55.

Davidson, W. N. (1916). *J. Econ. Ent.* **9**, 454-457.

——————— (1919). *Canad. Ent.* **51**, 235-239.

D'Herculais (1875 & 1881). Recherches sur L'Organisation et le développement des *Velucellcs* insectes diptères de la famille des Syrphides, Pts. I and II.

Fletcher, T. B. (1916). *Agric. Res. Inst. Pusa, Bull.* No. 59.

Greene, C. T. (1917). *Proc. Ent. Soc. Wash.* **19**, 152-155, Plate XIX, fig. 3.

Howlett, F. M. (1909). Indian Insect Life, 610-612.
Lundbeck, W. (1916). Diptera Danica. V.
Macquart, J. (1842). Diptera Exotica. II, ii.
Malloch, J. R. (1915). *Bull. Ill. State Lab. Nat. Hist.* **11**, 342-344.
Metcalf, C. L. (1911). *Ohio Nat.* **11**, 337-344.
——— (1912). *Ohio Nat.* **12**, 397-404, 477-488, 533-541.
——— (1913). *Ohio Nat.* **13**, 81-93.
——— (1917, 1). *Ohio, Biol. Surv. Bull.* **1**.
——— (1917, 2). *Maine Agric. Expt. Stat. Bull.* No. 263.
Riley, C. V. and Howard, L. O. (1888). Insect Life **1**, 5-8.
Smith, J. B. (1890). *N. J. Agric. Expt. Stat. Bull.* No. 72, 11-14.
——— (1891). *Canad. Ent.* **23**, 242-243.
Verrall, G. H. (1901). British Flies, VIII.

EXPLANATION OF PLATES

PLATE LX, *Baccha pulchrifrons* AUSTEN

a, The egg × 36; *b*, young larva × 24; *c*, mouth parts of the larva highly magnified; *d*, full-grown larva × 12; *e*, end view of the posterior respiratory appendage highly magnified; *f*, the pupa × 12; *g*, the adult fly, female × 6.

PLATE LXI, *Paragus serratus* FABRICIUS

a, The egg × 24; *b*, full-grown larva × 12; *c*, mouth parts of the larva highly magnified; *d*, end view of the posterior respiratory appendage highly magnified; *e*, the pupa × 12; *f*, the adult fly, female × 9.

PLATE LXII, *Sphærophoria javana* WIEDEMANN

a, The egg × 36; *b*, chorion of the egg highly magnified; *c*, young larva × 21; *d*, full-grown larva × 9; *e*, end view of the respiratory appendage highly magnified; *f*, the pupa × 12; *g*, the adult fly, female × 6.

PLATE LXIII, *Syrphus serarius* WIEDEMANN

a, Full-grown larva × 12; *b*, mouth parts of the larva highly magnified; *c*, end view of the posterior respiratory appendage highly magnified; *d*, the pupa × 9; *e*, adult fly, female × 6.

PLATE LXIV, *Syrphus balteatus* DE GEER

a, The egg × 30; *b*, chorion of the egg highly magnified; *c*, young larva × 30; *d*, mouth parts of the larva highly magnified; *e*, full-grown larva × 12; *f*, end view of the posterior respiratory appendage, highly magnified; *g*, the pupa × 12; *h*, the adult fly, female × 6.

PLATE LXV, *Syrphus confrater* WIEDEMANN

a, Full-grown larva × 6; *b*, the pupa × 8; c, the adult male fly × 5.

PLATE LXVI, *Syrphus isaaci* SP. NOV

a, Full-grown larva × 9; *b*, the pupa × 9; *c*, the adult fly, female × 6.

PLATE LXVII, *Helophilus bengalensis* WIEDEMANN

a, The egg × 26; *b*, chorion of the egg highly magnified; *c*, young larva × 6½; *d*, full-grown larva × 4; *e*, terminal portion of the respiratory tube highly magnified; *f*, the pupa × 4; *g*, pupal respiratory cornu × 24; *h*, a small spiracle of the pupal respiratory cornu highly magnified; *i*, the adult fly, female × 3.

STUDIES IN SURMA VALLEY RICES AND THEIR CLASSIFICATION.

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I. Introduction.

The Surma Valley in Assam comprises the districts of Sylhet and Cachar. It is a flat plain with heavy clay to clayey loam soils. The river banks being naturally higher, the interior is interspersed with numerous natural depressions, called *haors*, the margins of which are used for the cultivation of spring rice (*boro*). Rice is the staple crop and is the mainstay of ninety per cent. of the people.

The Government Rice Farm at Karimganj was started in 1914 with a view to study the rice crop and to recommend suitable high yielding varieties to the cultivators. The farm has been successful in so far that up-to-date twenty-two superior varieties including two hybrids have been recommended and distributed to the cultivators. The material dealt with in this publication is the result of observation and study by the writers on rice cultivated at the Karimganj Farm since 1921.

1. Rainfall.

The warm, moist climate of Surma Valley is very well suited to rice cultivation. The rainfall, which is most essential for the successful cultivation of paddy, is copious and well distributed. Occasional high floods damage the crop very badly. The normal monthly and annual rainfall of the different sub-divisions in Surma Valley is shown below :—

Table I.

The normal rainfall of the different sub-divisions of Surma Valley.

Locality	January	February	March	April	May	June	July	August	September	October	November	December	Total
Sylhet .	·58	1·40	6·15	13·75	20·87	30·91	27·27	25·22	21·71	9·41	1·23	·26	158·86
Karimganj .	·85	1·71	8·63	17·69	21·50	29·37	23·85	22·61	18·45	8·4	1·34	·47	154·88
Moulvibazar .	·48	1·42	4·52	11·39	17·28	20·37	17·21	16·05	13·05	6·45	1·17	·32	109·71
Habiganj . .	·45	1·24	4·53	9·68	15·42	19·74	15·82	14·23	11·78	5·90	·83	·27	99·29
Sunamgunj .	·36	1·28	5·10	11·45	20·77	40·80	47·04	46·85	31·44	10·14	·91	·17	216·31
Silchar . .	·75	2·18	7·53	13·96	15·19	20·75	17·45	19·00	14·39	6·55	1·43	·45	122·53
Hailakandi .	·84	1·77	7·20	13·24	15·69	20·23	17·29	17·05	12·7	6·61	1·24	·47	114·33
Average . .	·62	1·57	6·24	13·02	18·10	26·02	23·70	23·00	17·65	7·64	1·16	·34	139·06

The annual normal rainfall in Surma Valley ranges from 99·29 in. in Habiganj to 216·31 in. in Sunamganj, the average being 139·06 in. The average highest rainfall is obtained in June. The period from June to August is the most rainy

and is the best time for *sail* cultivation, which is the main crop of rice in Surma Valley.

2. Area and distribution of Surma Valley rice.

Rice covers an area of about seventy-three per cent. of the total area of crops sown in the Province. The crop forecast of 1930-31 for Assam shows that the estimated total area under summer and autumn rice is 816,900 acres, winter rice 3,417,200 acres, and spring rice, which is grown only in Sylhet district, 192,100. The following table shows the three years' average area under rice in Surma Valley as published in the crop forecasts of the Department of Agriculture, Assam, from 1928 to 1930 :—

District	Autumn rice	Winter rice	Spring rice	Rice seedlings	Total area under rice	Net area sown	Per cent. of area under rice
Sylhet . .	176,433	1,230,200	201,200	50,000	1,657,833	2,120,983	78·1
Cachar . .	26,800	212,300	..	12,500	251,600	334,153	75·3
Total .	203,233	1,442,500	201,200	62,500	1,909,433	2,455,136	77·8

In fact, about seventy-eight per cent. of the cultivated area in Surma Valley is under rice.

II. Agricultural classes of rice.

Various classes of rice are grown by the cultivators of Surma Valley including a large number of varieties suitable to different localities, depending mostly on the season and water requirements of the plants. They may briefly be described under four heads as follows :—

1. Summer rice.

The summer rice is generally known as broadcast *aus* and is grown on relatively higher areas. Its water requirement is comparatively less than that of other classes of rice. It is sown from March to May and harvested in June and July. It comprises three groups, *viz.*, *dumai*, *murali* and *chengri*. The *dumai* (two months) is equivalent to *shăithă* or *săthi* (sixty days) rice of Bengal. It is the earliest of all and takes about fifty-five days from sowing to flowering. It is sometimes sown in low-lying areas very early so that it may be harvested before the advent of the early flood. The *murali* and *chengri* are about ten days later than *dumai*, and are likewise grown on the high land and cultivated in

the same way as *dumai*. Under favourable conditions broadcast *aus* will yield up to fifteen maunds per acre.

2. Autumn rice.

The transplanted *aus* comes under the autumn rice. It is sown mostly in May transplanted after twenty to thirty days and harvested in August. It takes about seventy-five to eighty days from sowing to flowering and, owing to its longer period of growth and better cultural operations, its yield is higher than that of the broadcast *aus*.

3. Winter rice.

The winter rice comprises four classes, *viz.*, (*a*) *aman*, (*b*) *asra*, (*c*) *sail*, and (*d*) *birain*. The *aman** is a long-stemmed deep water rice having a prostrate stem which remains floating on water. After the water subsides, the stem falls flat on the mud giving out roots at the nodes and tillers freely. It grows well in deep water ranging from six to twelve feet or even more. It is sown broadcast in March and April and harvested in November and December. It gives a higher outturn (up to thirty-five maunds per acre) than any other winter rice.

The *asra*, as it is called in Surma Valley, is a shallow water *aman* which thrives well in three to six feet of water, *i.e.*, on comparatively higher land than *aman*, but lower than *sail*. It is sown broadcast from March to June and harvested at the same time as *aman*. It yields up to thirty maunds per acre.

The *sail* (transplanted *aman*) thrives well where the water does not rise above two feet. It is the most widely grown rice, and by far the best of all classes in quality. It is generally sown in June and July, transplanted from July to August and harvested in December. It gives an outturn of from twenty-five to thirty maunds per acre.

The glutinous rice is generally known here as *birain*. It is not grown on a commercial basis but only in limited areas. Most of the varieties are coarse and highly glutinous. Considering its cultural and other plant characteristics most of the varieties belong to the *sail* class, while there are some in *asra*, *aman* and *boro* (spring rice), but none has been found in the *aus* class. The cultivation of *birain* is exactly like that of the class of rice to which it belongs.

4. Spring rice.

The spring rice (*boro*) is a marsh-land rice grown on the margin of *beels* and *haors* (large natural depressions), which are left almost dry in the cold

* Experimental work on *aman* and *boro* (spring rice) has not yet been taken up in Assam.

weather. *Boro* is sown in November, transplanted from December to January and harvested in April and May. The outturn ranges from thirty-five to forty maunds per acre.

The description given above refers to cultivated rice only. It will not be out of place to mention here the wild rice (*O. sativa* var. *fatua*, Prain) locally known as *Jhora dhan* growing wild on the margin of tanks, ditches, *haors*, and is used for fodder.

III.—Characters of rice.

1. Varieties.

A large number of samples of rice of the *aus*, *sail*, *birain* and *asra* classes were collected for experimental purposes at the Karimganj Farm. Although in no way exhaustive, the collection fairly represents the Surma Valley rices. The total number of named varieties collected up-to-date is 369, of which 703 types were isolated. In addition 175 different samples have been obtained from other provinces in India, Burma and foreign countries, and are under observation at the Karimganj Farm.

The samples thus collected were named according to the versions of the cultivators from whom they were collected. Sometimes the same variety has been differently named in different localities. For example, one variety which has been named as Bherapawa in Cachar and Karimganj, is known as Neealu in North Sylhet and South Sylhet, and Khemu in Habiganj. Similarly, six different samples are named as Balam although they differ in character in many respects. Moreover, the varietal names of some of the samples differ slightly in the different localities of Surma Valley, such as Kartica (an early *sail* variety named after the month of *Kartic* when it is harvested) in Silchar, Kati Sail in Karimganj, Kartic Sail in North Sylhet and Katyabhog in Habiganj.

2. Flowering.

In a previous publication the writers [Mitra, Gupta and Ganguli, 1926] have shown that the flowering of *aus* is *periodically* fixed, *i.e.*, they will flower in a definite length of time more or less irrespective of their date of sowing. The number of days from sowing to flowering does not vary with the date of sowing but it may vary with the climatic conditions. For sample, an *aus* such as As. $^{1}\frac{36}{6}$ Tepi Dumai and M. $\frac{36}{30}$ Baurash Murali, will take fifty-five and sixty-six days respectively from sowing to flowering, no matter when they are sown. On the other hand, the flowering of the winter rice is *timely* fixed, *i.e.*, they will flower at a particular time between the months of October and November irrespective of their date of sowing.

For example, a *sail* such as S. 22 Latisail or S. 154 Georgesail, will flower in the fourth week of October whether they are sown in June or September. The number of days from sowing to flowering in winter rice will, therefore, directly vary with the date of sowing, as the time of flowering is almost fixed, but it may slightly vary with the climatic conditions.

The number of days from sowing to flowering varies from fifty-two to fifty-nine days in *dumai*, fifty-nine to sixty-six days in *murali* and *chengri*, and sixty-seven to eighty-five days in transplanted *aus*. The flowering period of *sail* and *asra* is longer and continues for a period of about thirty-four days commencing from the first week of October to the first week of November, and in *birain* it takes about a couple of weeks from the middle of October. Some of the types collected from Madras and Burma flower very late. They are transplanted at the same time as *sail*, but they flower in the last part of November, while most of the local *sail* flower by the fourth week of October and not later than the first week of November. The tables following [Tables II and III] will show the actual figures on flowering.

TABLE II.

Showing variation in the number of days from sowing to flowering (flowering periodically fixed).

Class of rice	No. of types under observation	Range of variation	Mode	Mean	Co-efficient of variability per cent.
Broadcast *aus*. . .	77	52-66 days .	63 days	61 days	6·11 ± ·33
Transplanted *aus* . .	50	67-85 „ .	Indefinite	75 „	5·48 ± ·37

TABLE III.

Showing variation in the date of flowering (flowering timely fixed).

Class of rice	No. of types under observation	Range of variation	Mode	Mean	Co-efficient of variability per cent.
Sail	321	2nd Oct.-4th Nov.	21st Oct.	22nd Oct.	3·79 ± 1·0
Birain .	75	15th Oct.-28th Oct.	Do.	23rd Oct.	2·03 ± ·11
Asra	101	5th Oct.-7th Nov.	Do.	22nd Oct.	3·57 ± ·17

The time of flowering varies slightly from year to year. The length of the growing period varies a good deal with the climatic conditions of the locality in which a particular variety is grown, as shown by Thadani [1928].

3. Opening of flowers.

Opening of rice flowers has been studied by Hector [1913], Parnell [1917], Bhide and Bhalerao [1927], Thadani [1928] and Sethi and Saxena [1930]. Observations were made on the same lines at the Karimganj Farm with Surma Valley rices and the results corroborate those of other observers in many respects. One *aus* and three *sail* types were studied at different times. Six ears of the *aus* and three ears of each of the *sail* types were taken together. The observations were made on two points, *viz.*, (*a*) the number of days required for the opening of all the flowers in the ear, and (*b*) the time of day when the opening takes place.

(*a*) The number of days required for the opening of all the flowers in the ear was found to vary from five to nine days, the smaller ears taking a shorter period. Flowering was most vigorous from the second to the fifth day in most cases and the maximum rate of flowering was obtained on the second or the third. (Appendix I, *a* and *b*).

(*b*) The time of day at which the rice flowers open varies with the climatic conditions, as has been rightly observed by Thadani [1928]. In the rainy days flowering begins late and continues even up to 3 P.M., whereas on sunny days it is finished before 12-30 P.M. In both *aus* and *sail* flowering was vigorous from 10 to 11 A.M., and had rain not intervened, most of the flowers would have completed opening by 11-30 A.M. It was also noticed that as the season advanced and got cooler flowering was delayed (Appendix I, *a* and *b*).

4. Colour of vegetative and floral parts.

The inheritance of colour combinations amongst the different types of Surma Valley rices has been studied in detail by the writers [Mitra, Gupta and Ganguli, 1928]. Separate groups were made in a broader sense overlooking the minor details. For example, there are different shades of colour in the leaf sheath, such as, light pink, pink, purple, deep purple and so on, but they were all grouped into one class as ' coloured '. Moreover, the location of colour differs in different types as with other characters. The colour combinations of Surma Valley rice has been analysed in the form of a chart of which a summary is given below.

Eight different groups of colour combinations are found in eighty-six types of broadcast *aus*, the majority (eighty) of which belong to two groups and the rest in six different colour patterns. Moreover, in fifty-one types of transplanted *aus* there

are fifteen groups of colour patterns. The real coloured types with distinctly coloured vegetative and floral parts are very rare in either broadcast or transplanted *aus*. The very slight pink or reddish colour in the leaf-sheath, apiculus and stigma is peculiar to *aus* and is very rarely found in other classes of rice. The reddish colour of the apiculus is found only in the lemma, while some of the bristles are only found coloured in the plumose stigma.

Out of a population of 378 types in *sail*, twenty-six different colour patterns have been noted of which the first nine groups include most of the types (359), while the rest are mostly in singles. Similarly, twenty-four different colour patterns have been observed in seventy-five types of *birain* and considering the number of types (sixty) in this group the colour patterns are numerous. Furthermore, 107 *asra* types have been grouped into twenty different combinations, the first six of which include the majority (ninety-one) of the types while the rest are mostly in singles. In fact combining the five classes of rice altogether seventy-four independent colour patterns have been noted from a total number of 703 isolated pure types in Surma Valley rice.

5. Leaf.

A typical rice leaf comprises the sheath, blade, ligule and auricles. In measuring the leaf, only the leaf-blade has been taken into consideration. The first leaf is a mere scale and the second one is also a scaly structure but a little larger than the first. It is the third leaf which has the shape of a typical leaf bearing the blade.

It has been observed that on the main culm there are about fourteen leaves in *aus* and nineteen in *sail*. The number of leaves on the main shoot is larger than that of the tillers, and the later the tiller comes out, the less is the number of leaves on it, although none has fewer than five or six. The length of the successive leaves is on the increase until the twelfth leaf in *aus* and the thirteenth or the fourteenth leaf in *sail*, after which there is a decrease in length. In *sail* the length of some of the intermediate leaves (eleventh to seventeenth) is approximately equal after which there is a decided fall. The flag or the boot leaf, which is the last leaf on each culm, is much shorter than some of its predecessors, but it is the broadest of all. In *aus* it is comparatively much larger than in *sail*. Except the very short ones, a leaf takes a little more than a week to complete its growth.

The rate of growth in leaves is also on the increase up to a certain limit, *i.e.*, up to the seventh week in both *aus* and *sail*, after which there is a gradual decrease with but slight variation until all growth is stopped in the eleventh week in *aus* and the nineteenth week in *sail*.

In order to find out the average variation in the area of leaf-blade, the third leaf from the flag which is the longest in rice was measured.

TABLE IV.

Showing variation in the area of the leaf-blade in rice.

Class of rice	No. of types under observation	Range of variation in sq. cm.	Mode	Mean	Co-efficient of variability per cent.
Broadcast *aus* . .	122	7-21	11	12·2	24·67±1·19
Transplanted *aus* . .	89	11-35	13	17·42	34·32±2·14
Sail	100	14-41	25	26·94	20·42±1·01
Birain	57	17-37	23-25	24·51	21·66±1·51
Asra	113	21-57	41	38·82	16·05±0·74

6. *Transformation of inner glume colour.*

There is a relation of inner glume colour between the flowering and the mature stages. Some of the observations on Surma Valley rices are noted below :—

(*a*) Green inner glume generally ripens yellow, *e.g.*, S. 22 (Latisail).

(*b*) Brownish inner glume ripens brown, *e.g.*, S. 36 (Larbong).

(*c*) Brown inner glume ripens deep brown, *e.g.*, S. 135 (Maina sail).

(*d*) Blackish inner glume ripens black, *e.g.*, S. 277 (Kalimekri).

(*e*) Purple or deep purple inner glume ripens black or deep black, *e.g.*, S. $^{2}\frac{2}{1}^{7}$ (Kalijira).

(*f*) Mottled grains show the mottled character in the early stage, *e.g.*, S. 20 (Jaria), S. 49 (Paikarchari), S. 23 (Terabali), etc.

There are undoubtedly exceptions in each one of the above types of colour change although they are few in number. Moreover, inner glumes which remain green for a long time suddenly turn black just before maturity, as in S. 248 (Pakikola). The mottled characters also sometimes develop from the green colour, as in S. 226 (Madhumadhav).

7. *Tillering.*

The rice plant has the capacity of producing a shoot from the axil of each leaf. But, except in deep water rice, tillers arise only from the base of the culm where the internodes are very short. In a well-developed plant all the culms appear to spring from a common point. The tillers are usually produced on alternate sides of the main shoot. These secondary shoots will in their turn produce tillers under favourable conditions.

Under normal conditions plants begin to tiller a week after transplanting, the highest number of tillers being obtained in the seventh week in *aus* and the eighth week in *sail*. Sufficient space all round the plant favours tillering, although some of the late tillers either die or flower too late.

TABLE V.

Showing variation in tillering of rice.

Class of rice	No. of types under observation	Range of variation	Mode	Mean	Co-efficient of variability per cent.
Broadcast *aus* . . .	39	1-3	2	2·36	22·03±1·75
Transplanted *aus* . .	28	2-4	4	3·54	16·10±1·48
Sail	116	3-12	6	6·45	25·74±1·21
Birain	56	2-11	5	5·3	2 ·68±1·92
Asra	42	5-14	7	8·19	26·74±2·10

Though tillering is a hereditary character, it varies greatly with climatic conditions and fertility of the soil. Tillers from ten normal plants were counted in each type whence the average per plant was calculated.

8. Straw.

(*a*) *Length of straw.*—The length of straw mostly varies with the supply of water and the fertility of the soil, although the individual length depends much upon the hereditary character of the different types. The longer types are generally more liable to lodge than the shorter ones, but the strength of straw is directly related to lodging. Deep water rices are all of lodging habit. The length of straw was measured about a fortnight after flowering, when the growth of the plant was stopped. An average measurement of five plants was taken in each case.

TABLE VI.

Showing variation in the length of straw of rice.

Class of rice	No. of types under observation	Range of variation	Mode	Mean	Co-efficient of variability per cent.
Broadcast *aus* . .	77	2 ft. 4 in.-3 ft. 4 in.	2 ft. 9 in.	2 ft. 8·58 in.	7·43±·57
Transplanted *aus* .	49	2 ft. 6 in.-3 ft. 8 in.	3 ft.	3 ft. 0·33 in.	9·51±·09
Sail . . .	272	2 ft. 8 in.-4 ft. 10 in.	3 ft. 8 in.	3 ft. 9·05 in.	9·16±·40
Birain . . .	57	2 ft. 10 in.-4 ft. 4 in.	3 ft. 4 in.	3 ft. 5·33 in.	9·31±·88
Asra . . .	56	4 ft. 8 in.-7 ft.	5 ft. 8 in.	5 ft. 9·86 in.	9·56±·70

The average length of straw varies from 2 ft. 4 in. in broadcast *aus* to 7 ft. in *asra*. The stem of *asra* grows with the rise of water, so it varies greatly with the water-level. The straw of *aman* is even longer as has been stated before.

(*b*) *Strength of straw.*—Rices with strong straw generally stand erect and have distinct advantage over those with weak straw which will invariably lodge. Very weak rices will lodge before flowering, while the less weak ones will lodge after flowering or before maturity. Lodging is a hereditary character but excessive vegetative growth or much water at the base during maturity may also cause lodging.

TABLE VII.

Showing variation in the percentage of weak and strong straw in rice.

Class of rice	No. of types under observation	Percentage of	
		Strong	Weak
Transplanted *aus*	73	55·0	45·0
Sail	373	73·6	26·4
Birain	55	66·7	33·3

Almost all the broadcast *aus* types are weak while those of *asra* and *aman* are well-known for their lodging and floating habit.

9. *Internode.*

Internodes at the base of a culm are very short, but they increase in length from below upwards. The number of internodes per culm varies with the length of the stem. The range of variation is from three to fifteen and is more in the case of deep-water *aman*.

TABLE VIII.

Showing variation in the number of internodes per culm in rice.

Class of rice	No. of types under observation	Range of variation	Mode	Mean	Co-efficient of variability per cent.
Broadcast *aus* . . .	67	3-5	4	3·8	12·63±0·74
Transplanted *aus* . . .	41	3-6	4	4·3	15·81±1·21
Sail	367	3-11	7	6·9	19·85±0·51
Birain	58	4-9	8	7·1	15·07±0·96
Asra	111	6-15	13	10·8	18·05±0·84

Comparing the above with the table for the length of straw it will be observed that the mean number of internodes increases with the increase of the mean length of straw in different classes of rice.

10. Exsertion of peduncle.

By " exsertion " is meant that portion of the peduncle which has emerged out of the sheath of the flag-leaf [Copeland, 1924]. In cases where the length of the peduncle is less than the sheath the exsertion is *nil*, and a portion of the panicle is also enclosed within the sheath. This is a hereditary character. Some of the types have no exsertion, while others have peduncles well exserted. In Surma Valley rices it varies from *nil* to 14·99 cm. Environmental conditions, such as temperature and water supply, also modify it to some extent. It has been observed that the panicles of rice plants, grown in earthen barrels and suffering from lack of water during the flowering time, did not come out fully. Some of the very late *sail* types, flowering in the month of January, showed the panicles partially emerged.

TABLE IX.

Showing variation in the length of exsertion of peduncle in centimeters.

Class of rice	No. of types under observation	Range of variation	Mode	Mean	Co-efficient of variability per cent.
		cm.	cm.		
Broadcast *aus* . . .	64	0·00—12·07	5·72	5·66	34·72±2·32
Transplanted *aus* . .	41	1·27—14·99	5·72	6·60	39·00±3·31
Sail	233	0·00—13·34	4·45	5·72	62·28±2·58
Birain	60	1·25—12·5	5·0	5·27	48·19±3·59
Asra	56	0·00—14·61	3·18	7·01	50·26±3·91

It has also been observed in transplanted *aus* and *sail* that the fine types have mostly very long exsertions of peduncle.

11. Awn.

The awn is an extension of the superior inner glume (lemma). Its presence causes considerable inconvenience during threshing and husking the rice. The length of awn varies greatly in different rices. Those which have one inch or longer awns have been designated as ' awned ' those with less than an inch long awns

as 'short awned', and those with quarter of an inch long or less as 'trace of awns' only.

Awn character is very variable. The length of awns varies greatly in the same plant and in the same ear. They may be present in all spikelets or only in the more apical ones.

TABLE X.

Showing variation in the percentage of awned and awnless types in rice.

Class of rice	No. of types under observation	Percentage of			
		Awned			Awnless
		Long awns	Short awns	Trace of awns	
Broadcast *aus* . . .	89	6·7	21·3	29·2	42·8
Transplanted *aus* . .	73	9·8	29·4	23·5	37·3
Sail	373	6·6	8·0	19·6	65·8
Birain	55	10·7	14·6	10·7	64·0
Asra	113	14·0	17·8	14·9	53·2
Average percentage . .	...	9·6	18·2	19·6	52·6

The percentage of long awned and awnless types is found to be the highest in *asra* and *sail* respectively, and, on an average, there are 9·6 per cent. long awned and 52·6 per cent. awnless types. The longest awns, such as that of Bherapowa or Khemu, are as long as three in. or a little more. A few Java types have about five in. long awns.

12. Panicle.

There are mainly two forms of panicle, *viz.*, the erect and the drooping. These are hereditary characters and are scarcely modified by environment. Most of the cultivated rices have drooping panicles. Those with erect panicles are generally short and poor yielders; they are very few in number. The branches of the panicle in wild rice are very spreading while most of the cultivated rices have rather close panicles.

Besides these characters, a panicle may be either compact, medium or loose with regard to the number of spikelets per unit area. This is also a hereditary character.

TABLE XI.

Showing variation in the percentage of different forms of panicle in rice.

Class of rice	No. of types under observation	Compact	Medium	Loose
		Per cent.	Per cent.	Per cent.
Broadcast *aus*	89	1·5	19·7	78·8
Transplanted *aus*	73	4·9	78·0	17·1
Sail	373	29·8	53·5	16·7
Asra	113	75·4	22·8	1·8

The percentage of compact types is found to be the least in broadcast *aus* and the most in *asra*, while that of the loose is just the reverse. Moreover, the percentage of medium types is the highest in transplanted *aus* and the lowest in broadcast *aus*. Although compact panicles have generally a larger number of grains, the types with this character are not necessarily high yielders as they may be poor in tillering.

(*a*) *Length of panicle.*—Like tillering, the length of panicle is also important in actual outturn. Panicles from five plants of each type were taken and their length measured in centimetres.

TABLE XII.

Showing variation in the average length of panicle of rice in centimetres.

Class of rice	No. of types under observation	Range of variation	Mode	Mean	Co-efficient of variability per cent.
		cm.	cm.		
Broadcast *aus* . .	77	14—27	19	19·12	11·34± ·88
Transplanted *aus* .	49	18—27	23	22·27	16·72±1·54
Sail	272	18—32	26	25·26	7·64± ·34
Birain . . .	57	18—28	23	23·95	10·43± ·67
Asra . . .	56	18—28	24	23·46	6·98± ·51

The average length of panicle varies from 14 to 32 cm. The length of the longest panicle has been found to be as much as 40 cm. in a *birain* and the shortest 11·8 cm. in a broadcast *aus*. A few Java types possess very long panicles (about 42 cm.).

(*b*) *Number of branches per panicle.*—The branches of the panicle, like the panicle itself are also erect or drooping. As a matter of fact, they are mainly responsible for the form of a panicle. The number of branches per panicle varies from six to sixteen in Surma Valley rice. It has a correlation with the length of panicle or the number of spikelets per panicle as will be discussed later.

TABLE XIII.

Showing variation in the number of branches per panicle in rice.

Class of rice	No. of types under observation	Range of variation	Mode	Mean	Co-efficient of variability per cent.
Broadcast *aus*	73	6—10	8	7·92	11·74±·66
Transplanted *aus*	50	6—11	8	8·10	10·25±·70
Sail	269	6—16	11	11·36	21·57±·59
Birain	57	8—14	9	10·54	13·85±·89
Asra	56	8—16	13	12·89	13·65±·69

(*c*) *Number of spikelets per panicle.*—Spikelets were counted from the panicles of the same five plants from which the length of panicle was measured. Although a hereditary character, the number of spikelets varies a good deal even in the same plant, while variation between different types is very well marked.

TABLE XIV.

Showing variation in the number of spikelets per panicle in rice.

Class of rice	No. of types under observation	Range of variation	Mode	Mean	Co-efficient of variability per cent.
Broadcast *aus*	77	40—160	70	68·57	27·07±1·57
Transplanted *aus*	51	50—150	80	94·51	23·36±1·63
Sail	269	60—396	150	166·54	31·69±1·01
Birain	57	60—200	120	122·81	26·49±1·78
Asra	56	100—280	200	185·0	21·06±1·40

In *asra* the panicles are compact and contain a larger number of grains than in any other class, although their mean length is less than either in *sail* or *birain*. The

panicles of broadcast *aus* are, on the other hand, loose and short, and contain less grains. The average number of grains per panicle varies from 40 in broadcast *aus* to 396 in *sail*. In a single panicle the largest number of grains (477) has been found in *sail* and the smallest (twenty) in broadcast *aus*. As reported by Jacobson [1916] the number of spikelets per panicle in Philippine rices varies from 50 to 478, while Thadani [1928] found it to range from 92 to 303 among Sind rices.

13. Size of spikelets.

The size of spikelets has been classified in various ways by different writers of which resume is given below :—

(*a*) As regards shape of unhusked grains, Kikkawa [1912] distinguishes between the following :—

(1) Long grain, in which the length is more than twice the breadth.

(2) Short grain, where the length is less than twice the breadth.

(3) Slender grain, where the length is greater than three times the breadth.

He then sub-divides each of the above three into large, medium and small grains.

(*b*) Graham [1913] divides spikelets of rice into four classes as follows :—

(1) Long spikelets, when the length is more than four times the breadth.

(2) Fine spikelets, when the length is more than three times the breadth.

(3) Coarse spikelets, when the length is more than twice the breadth.

(4) Round spikelets, when the length is less than the breadth.

(*c*) Beale [1927] divides the varieties of rice found in Lower Burma into five main groups according to size and shape of spikelets, as shown below.

	Length, mm.	Ratio of length to breadth
1. Emata	Over 9·4	Over 3·3
2. Letywezin	8·40 to 9·8	2·8 to 3·3
3. Ngasein	7·75 to 9·0	2·4 to 2·8
4. Medon	7·35 to 8·6	2·0 to 2·4
5. Byat	9·0 to 11·25	2·25 to 3·4

(*d*) In the United States [Copeland 1924] three groups of "kernels" are recognized :

(1) Short, less than 6 mm. long.

(2) Medium, 6 to 7 mm.

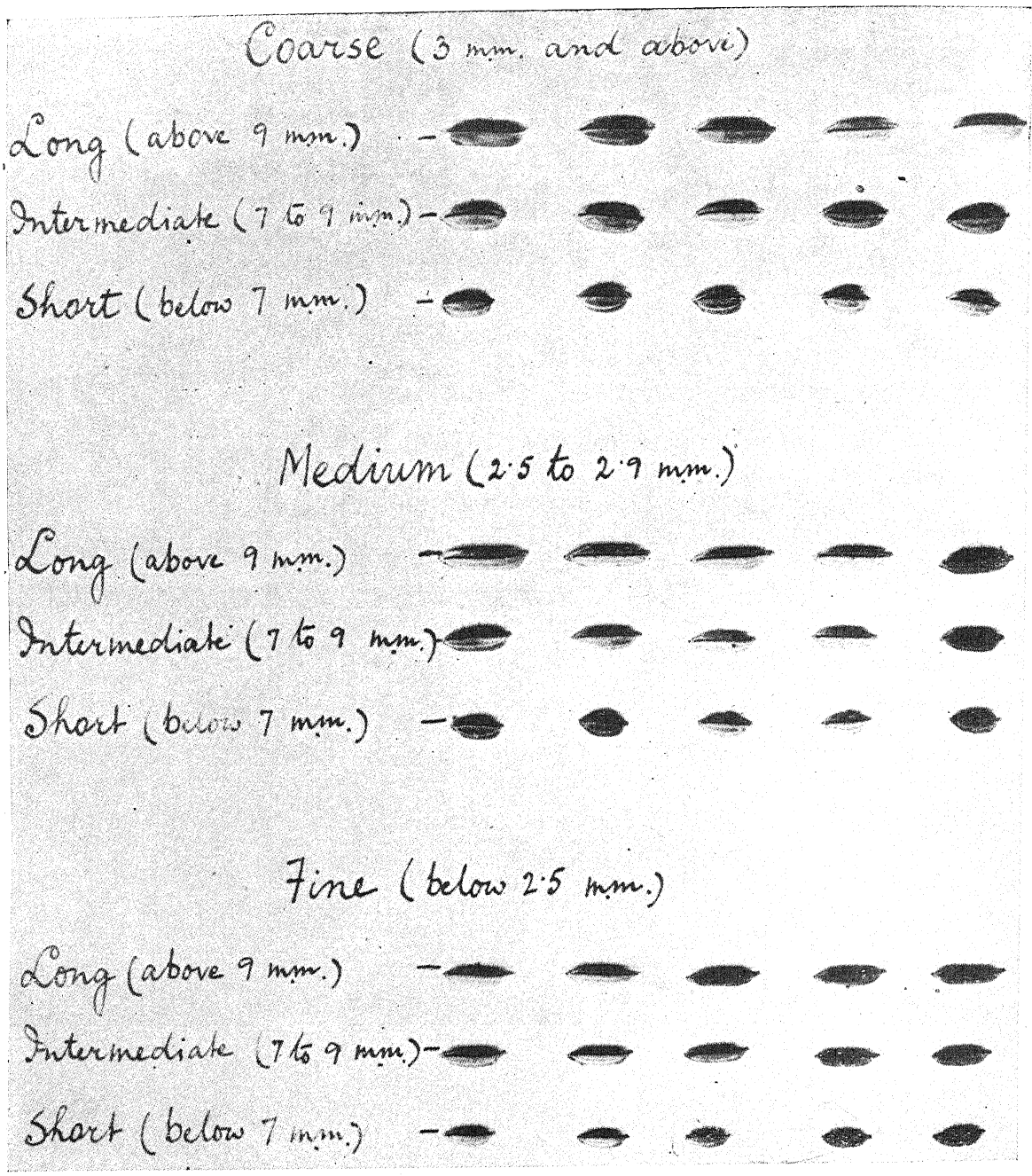
(3) Long, 7 mm. or longer.

To these Copeland [1924] has added two more.

(4) Very short, less than 5 mm.

(5) Very long, more than 8 mm.

PLATE LXVIII.



Size and shape of unhusked grains (coarse, medium or fine refers to breadth, and long, intermediate or short refers to length of grains).

(*e*) Thadani [1928] has classified the Sind rices with regard to size and shape of unhusked grains as follows :—

(1) Long grained rice, length above 8·9 mm.
(2) Medium grained rice, length between 7·9 and 8·9 mm.
(3) Short grained rice, length below 7·9 mm.

With regard to the breadth of unhusked grains the Sind varieties were classified as follows :—

(1) Rices with slender grains, breadth less than 2 mm.
(2) Rices with oval grains, breadth 2 to 2·5 mm.
(3) Rices with flat grains, breadth more than 2·5 mm.

(*f*) Sethi and Saxena [1930] divided 135 varieties of U. P. rices into fifteen different groups according to the colour of kernel as well as the size and shape of unhusked grains as follows :—

Division I, white grain and Division II, coloured (red) grain. Both the divisions are grouped as follows :—

Group A.—Slender grained, when the length is thrice or more than three times the breadth.

Group B.—Long grained, when the length is more than twice the breadth but less than thrice.

Group C.—Short grained, when the length equals twice or is less than twice the breadth.

Each of the above groups is again sub-divided into large, medium and small.

The size of spikelets varies considerably in different types of Surma Valley rices. The variation within the type is rather limited. The length, breadth and thickness of the unhusked grains (as placed on the table) were measured with a micrometer scale. Average of ten unhusked grains, selected at random was taken in each type. In determining the coarseness or fineness the breadth of the unhusked grains counts most, while the length adds to its intensity. The following classification has been found convenient to suit popular ideas about the size and shape of unhusked grains of Surma Valley rices :—

Breadth	Length
Coarse — 3 mm. and above.	Long — above 9 mm.
Medium — 2·5 mm. to 2·9 mm.	Intermediate — 7 mm. to 9 mm.
Fine — below 2·5 mm.	Short — below 7 mm.

To be elaborate the following may be added to the above :—

Very coarse — 3·5 mm. and above.	Very long — above 10 mm.
Very fine — below 2 mm.	Very short — below 6 mm.

It may be noted here that a coarse, medium or fine spikelet may be either long, intermediate or short and *vice versâ* (Plate LXVIII).

TABLE XV.

Showing variation in the length, breadth and thickness of spikelets.

Class of rice	No. of types under observation	Length	Breadth	Thickness
		mm.	mm.	mm.
Broadcast *aus*	89	6·28-8·48	2·5-3·16	1·84-2·16
Transplanted *aus*	73	6·62-9·12	1·8-2·92	1·7-2·12
Sail	373	5·56-10·7	1·98-3·52	1·3-2·5
Birain	55	7·24-10·94	2·14-3·74	1·74-2·4
Asra	113	6·6-11·3	2·6-3·5	1·9-2·5

The above table shows that the shortest spikelets are found in *sail* and the longest in *asra*, while the finest are found only in transplanted *aus* and *sail*, and the coarsest in *birain*.

TABLE XVI.

Showing variation in the percentage of types having different sizes of spikelets.

Class of rice	No. of types under observation	Breadth in mm.	Length			Total percentage
			Below 7 mm.	7 to 9 mm.	Above 9 mm.	
		mm.				
Broadcast *aus*	89	Below 2·5	*Nil*	1·3	*Nil*	1·3
		2·5 to 2·99	6·7	77·4	*Nil*	84·1
		Above 3	5·3	9·3	*Nil*	14·6
Transplanted *aus*	73	Below 2·5	*Nil*	22·0	*Nil*	22·0
		2·5 to 2·99	6·0	70·0	2·0	78·0
		Above 3	*Nil*	*Nil*	*Nil*	*Nil*
Sail	373	Below 2·5	9·2	10·4	2·1	21·7
		2·5 to 2·99	8·6	31·3	8·3	48·2
		Above 3	6·3	22·6	1·2	30·1
Birain	55	Below 2·5	*Nil*	1·8	1·8	3·6
		2·5 to 2·99	*Nil*	21·8	25·5	47·3
		Above 3	*Nil*	41·8	7·3	49·1
Asra	113	Below 2·5	*Nil*	*Nil*	*Nil*	*Nil*
		2·5 to 2·99	1·0	24·0	12·0	37·0
		Above 3	*Nil*	56·5	6·5	63·0
Total average percentage	...	..	8·6	78·0	13·4	..

The percentage of fine spikelets is *nil* in *asra*, very few in *birain* and broadcast *aus*, and the highest in transplanted *aus* and *sail*, while *asra* has the highest percentage of coarse spikelets. The total average percentage shows the excess of intermediate spikelets to a great extent. Short spikelets are not met with in *birain* and *asra*, but found only to a small extent in *aus* and about 22 per cent. in *sail*. Long spikelets are rarely found in *aus* while they occur mostly in *birain*, being about 35 per cent. on average.

14. Kernel.

(*a*) *Size of kernel*—The size of kernel corresponds to a large extent to the size of spikelets with a slight variation.

TABLE XVII.

Showing variation in the size of kernel in ten types of rice.

Types of rice	Spikelet			Kernel			Difference		
	Length	Breadth	Thickness	Length	Breadth	Thickness	Length	Breadth	Thickness
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
D. $\frac{138}{6}$ Tepi Dumai .	7·56	2·64	1·84	5·30	2·26	1·70	2·26	·38	·14
M. $\frac{36}{30}$ Baurash Murali .	7·86	2·66	1·96	5·60	2·34	1·80	2·26	·32	·16
As. 1 Lal aus . .	8·66	2·64	1·94	6·34	2·22	1·76	2·32	·32	·18
As. 3 Basmati . .	7·32	2·60	1·86	5·04	2·24	1·74	2·28	·36	·12
S. 22 Latisail . .	8·60	3·00	2·20	6·02	2·60	1·96	2·58	·40	·24
S. 300 Dighallata .	9·14	2·00	1·72	6·26	1·82	1·56	2·88	·18	·16
S. $\frac{227}{1}$ Kalijira . .	6·68	2·32	1·70	4·50	2·00	1·50	2·18	·32	·20
B. 16 Puspabirain .	10·94	2·92	2·00	7·52	2·30	1·78	3·42	·62	·22
Ar. 28 Birpak . .	8·70	3·40	2·30	6·20	3·00	2·10	2·50	·40	·20
Ar. 64 Joalbhanga .	11·30	2·80	2·10	8·20	2·40	1·90	3·10	·40	·20
Average . . .	8·68	2·70	1·96	6·10	2·32	1·78	2·58	·38	·18

The variation in the column of difference is from 2·18 to 3·42 in length, 0·18 to 0·62 in breadth, and 0·12 to 0·24 in thickness which is the least variable.

(*b*) *Consistency of kernel.*—Consistency, *i.e.*, the hardness or softness of kernel can be ascertained by cutting midway across the kernel. Those showing a translucent surface are hard or corneous and those showing a white opaque surface are soft. The latter are glutinous rice and they belong to the *birain* class. Among the non-glutinous rice some are entirely translucent while some have opaqueness in the centre or in the abdomen. It has been observed that most of the coarse rices are abdominal opaque while the majority of the fine are translucent.

TABLE XVIII.

Showing variation in the percentage of translucent and abdominal opaque rices in relation to coarseness or fineness of grains.

Class of rice	No. of types under observation	Translucent			Abdominal opaque		
		Coarse	Medium	Fine	Coarse	Medium	Fine
Broadcast *aus*	89	..	..	..	14·7	85·3	..
Transplanted *aus*	73	..	6·4	6·4	..	78·7	8·5
Sail	373	4·1	20·3	17·5	30·0	17·8	0·3
Asra	108	1·1	11·2	..	61·8	25·9	..
Total average	..	2·7	21·1	11·4	30·5	33·4	0·9

Out of 33·2 per cent. of coarse rice only 2·7 per cent. are translucent and the rest are abdominal opaque, while out of 12·3 per cent. of fine rice only 0·9 per cent. are abdominal opaque, *i.e.*, 8·1 per cent. of the coarse rice are translucent and 7·3 per cent. of the fine rice are abdominal opaque. Altogether 35·2 per cent. of the Surma Valley rice have been found to be translucent and 64·8 per cent. abdominal opaque. There is no translucent rice in broadcast *aus*. In transplanted *aus* and *asra* the percentage of translucent rice is 12·8 and 12·3 respectively. About 52 per cent. of *sail* is translucent which is thus decidedly a better class of rice than others.

(*c*) *Colour of kernel.*—The colour of kernel in different types of rice is either red, white or amber.

TABLE XIX.

Showing variation in the percentage of colour of kernel in rice.

Class of rice	No. of types under observation	Percentage of colour of kernel		
		Red	White	Amber
Broadcast *aus*	89	97·7	2·3	*Nil*
Transplanted *aus*	73	82·4	17·6	*Nil*
Sail	373	10·3	86·8	2·9
Birain	55	50·7	49·3	*Nil*
Asra	113	48·7	42·0	9·3
Total average	..	58·0	39·6	2·4

Except two, all the types in broadcast *aus* have red kernels while most of the types in transplanted *aus* are also red. The percentage of white kernel in *sail* is very high in relation to red and in *birain* they are almost equal in number. In *asra*, red kernel is slightly more frequent than white kernel. The amber colour of kernel is found only in *sail* and *asra* in low percentages.

15. Shedding habit.

"Tightness of grains" is a factor of some economic importance, and it is invariably a hereditary character. As noted by Copeland [1924] some of the rices become looser as they approach ripeness, while some are tighter when fully ripe than shortly before that time, but no regular study has been made of this character. Some of the rices in Surma Valley, which shed their grains easily, are known as *saiamora*. They are found mostly in *sail* and in a few *aus* rices. They, as a class, shed so badly on maturity that they have to be harvested before the usual ripening time to avoid loss of grains in the field. On the other hand, there are others like Latisail, Mulasail, etc., which are so tight that it is not possible to thresh out all the grains unless subjected to severe threshing. However, the rices with the medium character occur in large numbers. In Surma Valley, where harvesting is done by hand, and threshing by bullocks, high yielding types with moderate tightness are mostly desired.

16. Maturity.

In studying a large number of Philippine varieties of rice Crisostomo [1915] found that the time from flowering to maturity varies from eleven to sixty-nine days, the average being about thirty-three days. Sind rices have been found by Thadani [1928] to vary from twenty-four to fifty-three days, while at Larkana the variation was from ten to eighty days. In Surma Valley, such extreme variations are not met with. In *aus* it varies from twenty-five to thirty days and in *sail* and *asra* from thirty to forty days. But some of the types obtained from Burma, Madras and Philippine Islands took forty-five to fifty days to mature.

The problem that confronts the cultivators of Surma Valley in point of maturity lies in the fact that a major portion of this area is flooded every year during the rains. The cultivators are sometimes compelled to harvest their *aus* crop a few days earlier than the usual time for fear of the crop being submerged by flood or stagnant rain water which often does considerable damage to the crop. On the other hand, in the winter season the harvesting of the *sail* crop is sometimes continued late owing to the scarcity of labour.

In order to find out whether the percentage of germination or weight of grains are affected by this early or late harvesting, data were collected carefully for three years. One variety each of broadcast and transplanted *aus*, *sail*, and *asra* were selected for the purpose. Ten ears of each variety were collected on every alternate day from the tenth day of flowering up to the fiftieth day. 500 grains of each day's collection were weighed and the germination of 100 grains were tested.

(*a*) *Weight of unhusked grains.*—In both broadcast and transplanted *aus*, the weight of unhusked grains reaches its maximum on the twenty-sixth day and practically remains the same with a slight variation up to the fiftieth day (Appen-

dix II). In *sail* it reaches the maximum on the thirtieth day and in *asra* on the thirty-fourth day. No marked rise or fall is noticed up to the fiftieth day in either case. Continuous drought or heavy rains may cause the grains to mature early or late respectively to a certain extent.

(*b*) *Percentage of germination.*—It has been observed that more than 90 per cent. germination is obtained on the thirtieth day in broadcast *aus*, thirty-second day in transplanted *aus* and *sail*, and thirty-eighth day in *asra*. The germination does not deteriorate even if the crop remains in the field up to the fiftieth day under dry conditions (Appendix II). But, on the contrary, if the crop lodges in the standing water below, most of the grains will either germinate as in *aus* or lose their germinating capacity as in *sail* and *asra*.

The above results indicate that the mature grains attain the average weight on the twenty-sixth day in *aus*, thirtieth day in *sail*, thirty-fourth day in *asra*, while 90 per cent. of germination is obtained on the thirtieth day in broadcast *aus*, thirty-second day in transplanted *aus* and *sail*, and thirty-eighth day in *asra*.

17. Hull.

Rice grains are enclosed by the inner glumes, lemma and palea, which together constitute the hull. Both the size and thickness of the hull contribute to its total weight. Ten types of each of the different classes of rice were examined and 200 grains of each type were weighed with and without the husk. The percentage of hull was found to vary from 8·0 to 41·9 with an average of 23·6. Stok showed the percentage of hull to range from 15·8 to 23·0 in fifty varieties tested at Buitenzorg [Copeland, 1924]. In Sind rice Thadani [1928] found it to vary from a little over 18 to 22 in different varieties.

TABLE XX.

Showing variation in the percentage of hull in rice.

Class of rice	No. of types under observation	Average weight of grains in grms.	Average weight of kernel in grms.	Percentage of hull.
Broadcast *aus*	10	4·23	3·35	20·29
Transplanted *aus*	10	3·75	3·00	20·63
Sail	10	3·69	2·78	24·90
Birain	10	5·21	3·87	26·11
Asra	10	5·15	3·81	26·00
Total average	..	4·40	3·36	23·58

As the hull is a total waste in the grain, a low percentage of it is a distinctive quality in milling the rice. No definite relation could be observed between the size of grain and the percentage of hull, but the husks of the *aus* types are thin and those of *asra* and *birain* are thicker and consequently heavier as is shown in Table XX above.

18. Yield.

The yield of rice usually depends on the normal vegetative growth and the normal distribution of rainfall, the latter of which is the most important. It also depends on the length of straw, tillering, length of ears and the number of grains per ear. The period of growth as well as the fertility of the soil also play an important part. It has been observed that tillering and length of straw have a relation to yield. This is discussed later.

Like other characters yield is also a hereditary character although the environmental conditions affect the yield to a large extent. When grown under similar conditions some of the types will yield better than others, while there are some that will thrive in a particular water-level or in a particular kind of soil.

TABLE XXI.

Showing variation in yield in tolas *per 100 plants of each type.*

Class of rice	No. of types under observation	Range of variation	Mode	Mean	Co-efficient of variability per cent.
Broadcast *aus*	39	11-32	21	19·38	22·29±1·77
Transplanted *aus* . . .	28	20-47	35	34·68	18·83±1·75
Sail	116	60-170	130	123·36	18·08± ·82
Asra	42	160-420	270	251·9	22·71±1·75

The yield varies from 11 *tolas* in broadcast *aus* to 420 *tolas* in *asra*. It may be noted here that single seedlings were transplanted in each case.

IV. CORRELATION OF CHARACTERS IN RICE.

Correlation of characters in rice has been studied by Bhide and Bhalerao [1927] and Sethi and Saxena [1930]. While studying the above characters of the Surma Valley rices, various degrees of correlation both positive and negative were noticed

between different characters among the types of Surma Valley rices which may be summarised as follows :—

TABLE XXII.

Showing correlation between number of spikelets per panicle and length of the panicle.

Class of rice	No. of types under observation	Mean No. of spikelets	Mean length of the panicle in cm.	Co-efficient of correlation	Remarks
Broadcast *aus* . .	77	68·57	19·09	0·61±·05	Correlation close
Transplanted *aus* .	51	94·51	22·23	0·32±·08	,, appreciable
Sail	269	166·54	24·76	0·39±·03	Ditto
Birain . . .	57	122·81	23·95	0·32±·08	Ditto
Asra	56	185·00	23·46	0·68±·05	Correlation close

The correlation between the number of spikelets per panicle and the length of the panicle is close in broadcast *aus* and *asra* while in other cases it is appreciable.

TABLE XXIII.

Showing correlation between number of spikelets and number of branches per panicle.

Class of rice	No. of types under observation	Mean No. of spikelets	Mean No. of branches	Co-efficient of correlation	Remarks
Broadcast *aus*. . .	73	75·07	7·92	0·48±·06	Correlation fairly close
Transplanted *aus* . .	50	98·20	8·10	0·30±·08	Correlation appreciable
Sail	269	166·54	11·36	0·32±·04	Ditto
Birain	57	122·81	10·54	0·77±·04	Correlation close
Asra	56	185·00	12·89	0·77±·04	Ditto

The correlation between the number of spikelets and the number of branches per panicle is quite close in *birain* and *asra*, fairly close in broadcast *aus* and appreciable in others.

TABLE XXIV.

Showing correlation between length of panicle and length of straw.

Class of rice	No. of types under observation	Mean length of panicle in cm.	Mean length of straw in inches	Co-efficient of correlation	Remarks
Broadcast *aus*. . .	77	19·12	32·58	0·19±·07	Correlation slight
Transplanted *aus* . .	49	22·27	36·33	0·28±·09	Correlation rather appreciable
Sail	272	25·26	45·05	0·41±·03	Correlation fairly close
Birain	57	23·95	41·33	0·36±·08	Correlation appreciable
Asra	56	23·46	69·86	0·11±·09	Correlation slight

The above results show that correlation between the length of panicle and the length of straw is fairly close in *sail*, appreciable in *birain*, rather less appreciable in transplanted *aus* and slight in others.

TABLE XXV.

Showing correlation between yield and tillering of rice.

Class of rice	No. of types under observation	Mean yield in grms. per 100 plants	Mean No. of tillers per plant	Co-efficient of correlation	Remarks
Broadcast *aus*. . .	39	219·78	2·36	0·22±·10	Correlation rather appreciable
Transplanted *aus* . .	28	393·27	3·54	0·39±·10	Correlation appreciable
Sail	116	1,398·90	6·45	0·25±·06	Correlation rather appreciable
Asra	42	2,856·55	8·19	0·22±·09	Ditto

The correlation between the yield and the tillering is quite appreciable in transplanted *aus* and rather less appreciable in others.

TABLE XXVI.

Showing correlation between yield and length of straw.

Class of rice	No. of types under observation	Mean yield in grms. per 100 plants	Mean length of straw in inches	Co-efficient of correlation	Remarks
Broadcast *aus*. . .	39	219·78	32·44	0·42±·04	Correlation fairly close
Transplanted *aus* . .	28	393·27	36·89	0·32±·02	Correlation appreciable
Sail	116	1,398·90	51·53	—0·10±·06	Correlation appreciable and slight
Asra	42	2,856·55	80·29	0·07±·10	Correlation negligible

The amount of correlation between the yield and the length of straw varies a good deal in different classes of rice. In *sail* it is negative while in others positive. Correlation is fairly close in broadcast *aus*, appreciable in transplanted *aus*, slight in *sail* and negligible in *asra*.

TABLE XXVII.

Showing correlation between yield and length of panicle.

Class of rice	No. of types under observation	Mean yield in grms. per 100 plants	Mean length of panicle in cm.	Co-efficient of correlation	Remarks
Broadcast *aus*. . .	39	219·78	19·05	0·25±·10	Correlation rather appreciable
Transplanted *aus* . .	28	393·27	22·43	—0·04±·10	Correlation negligible
Sail	116	1,398·90	26·16	—0·14±·05	Correlation rather slight
Asra	42	2,856·55	25·19	0·37±·09	Correlation appreciable

The amount of correlation also varies a good deal in yield and length of panicle. It is appreciable in *asra*, rather less appreciable in broadcast *aus*, slight in *sail* and negligible in transplanted *aus*. Moreover, correlation is positive in broadcast *aus* and *asra* and negative in others.

TABLE XXVIII.

Showing correlation between yield and number of days from sowing to flowering.

Class of rice	No. of types under observation	Mean yield in grms. per 100 plants	Mean No. of days from sowing to flowering	Co-efficient of correlation	Remarks
Broadcast *aus*. . .	39	219·78	59·0	0·12±·10	Correlation slight
Transplanted *aus* . .	28	393·27	74·0	—0·14±·10	Ditto
Sail	116	1,398·90	136·57	0·23±·06	Correlation rather appreciable
Asra	42	2,856·55	174·85	0·35±·09	Correlation appreciable

The correlation between yield and the number of days from sowing to flowering is negative in transplanted *aus* and positive in other cases. It is appreciable in *asra*, rather appreciable in *sail* and slight in both broadcast and transplanted *aus*.

TABLE XXIX.

Showing correlation between tillering and length of panicle.

Class of rice	No. of types under observation	Mean No. of tillers per plant	Mean length of panicle in cm.	Co-efficient of correlation	Remarks
Broadcast *aus*. . .	39	2·32	19·05	—0·23±·10	Correlation rather appreciable
Transplanted *aus* . .	28	3·48	22·43	—0·25±·12	Ditto
Sail	116	6·45	26·16	—0·38±·05	Correlation appreciable
Asra	42	8·19	25·19	—0·11±·10	Correlation slight

From Table XXIX it will be seen that the correlation between tillering and length of panicle is negative in all cases. The correlation is appreciable in *sail*, rather less appreciable in broadcast *aus* and transplanted *aus*, and slight in *asra*.

TABLE XXX.

Showing correlation between tillering and length of straw.

Class of rice	No. of types under observation	Mean No. of tillers per plant	Mean length of straw in inches	Co-efficient of correlation	Remarks
Broadcast *aus* . .	39	2·32	32·44	—0·27±·10	Correlation rather appreciable
Transplanted *aus* . .	28	3·48	36·89	0·19±·12	Correlation slight
Sail	116	6·45	51·53	—0·41±·05	Correlation fairly close
Asra	42	8·19	80·29	—0·01±·10	Correlation negligible

The correlation is negative in all cases except in transplanted *aus*. It is fairly close in *sail*, rather less appreciable in broadcast *aus*, slight in transplanted *aus* and negligible in *asra*.

The girth of the stem near the ground level is definitely correlated to the length of straw and panicle as has been observed by Joshi and Gadkari [1923]. The bigger the girth the longer is the stem or panicle. Tables XXXI and XXXII will show the degree of correlations in each case.

TABLE XXXI.

Showing correlation between girth of internode and the length of straw.

Class of rice	No. of types under observation	Mean girth in cm.	Mean length of straw in cm.	Co-efficient of correlation	Remarks
Sail	206	2·08	124·32	0·51±·03	Correlation close

The correlation here is close.

TABLE XXXII.

Showing correlation between girth of internode and the length of panicle.

Class of rice	No. of types under observation	Mean girth in cm.	Mean length of panicle in cm.	Co-efficient of correlation	Remarks
Sail	206	2·08	26·0	0·44±·04	Correlation fairly close

The correlation here is fairly close.

V. Classification.

The need of botanical classification of rice has long been felt from all quarters. The agricultural classification, as described before, mainly depends on the water requirements of plants and the time of flowering and ripening of grains. This method of classification is no doubt of great economic value but it does not satisfy the requirements of scientific classification. In fact, in such a vast area where rice is cultivated under various conditions, it is rather difficult to classify it without any definite standard. Some fixed characters may, therefore, be chosen as has been done by both Kikkawa [1912] and Graham [1913]. They pointed out clearly the defects of agricultural classification and have advocated the characters of grains as the only basis of botanical classification. Beale [1927], Thadani [1928] and Sethi [1930] have worked out a system of classification on this basis in Burma, Sind and United Provinces respectively. In our work with the Surma Valley rice we have also tried to classify the isolated types according to unhusked grain characters alone, as has been stated, when dealing with the size of spikelets.

Apart from the size of spikelets, the awn, consistency of endosperm, and colour of kernel and inner glume have been used as a basis for this classification. The kernel may be either white, red or amber, while the inner glume may have a wide range of variation. In fact, the inner glume colour is the most prominent character that attracts the eye of the observer in a sample of rice. It has been divided into four groups, *viz.*, (*i*) yellow, (*ii*) brown, (*iii*) black, and (*iv*) mottled, which are the main colour characters of Surma Valley rice. It may safely be said that the basic colour of the inner glume is green at the outset and changes to yellow unless any other colour develops on it. The other two colours, *viz.*, the brown and the black, develop on this yellow basic colour during maturity. This is evident from the fact that even in a brown or black coloured rice the undeveloped spikelets or the empty glumes always show the yellow as the basic colour with a shade of brown or black, as the case may be. Where the development of brown or black colour is not complete in the mature grain, it shows yellow colour in patches, which has been brought under one group—the mottled. Moreover, the intensity of any colour, such as deep, medium or light has been taken together in the same group in each case, while all those showing the combination in patches or in stripes have been taken as mottled.

In order to facilitate the arrangement of individual types in different groups, the simple alphabetical characters have been used in the key to the scheme of classification with a view to name them definitely, as has been done in arranging the rice specimens of Surma Valley in the botanical laboratory at Karimganj. These alphabetical characters show the respective grain characters of each individual variety as well. For example, Latisail (S. 22), Georgesail (S. 153), and Basmati (As. 3) have been designated as $A_1\ B_1\ C_2\ D_2\ E_1\ F_1$, $A_1\ B_1\ C_1\ D_2\ E_1\ F_2$ and $A_1\ B_1\ C_2$

D_2 E_1 F_1 respectively as shown in the key below and this has been followed in the case of all the other known types of rice in Surma Valley.

It may also be pointed out here that seventeen different characters of the unhusked grain have been dealt with in this classification, which may be combined with each other to fall into 432 possible combinations of which the majority are wanting. In the following chart 703 types of rice have been classified according to unhusked grain characters alone.

Key to the classification of Surma Valley rice.

Kernel				Inner glume	No. of types
		A_1—Non-glutinous.			
B_1—Awnless	C_1—Coarse	D_1—Long	E_1—White	F_1—Yellow	3
				F_4—Mottled	2
			E_2—Red	F_2—Brown	1
				F_3—Black	2
		D_2—Intermediate	E_1—White	F_1—Yellow	29
				F_2—Brown	9
				F_3—Black	4
				F_4—Mottled	18
			E_2—Red	F_1—Yellow	21
				F_2—Brown	1
				F_4—Mottled	11
			E_3—Amber	F_1—Yellow	8
				F_4—Mottled	1
		D_3—Short	E_1—White	F_1—Yellow	7
				F_4—Mottled	13
			E_2—Red	F_1—Yellow	2
	C_2—Medium	D_1—Long	E_1—White	F_1—Yellow	8
				F_2—Brown	12
			E_2—Red	F_1—Yellow	2
				F_2—Brown	2

Kernel				Inner glume	No. of types
B_1—Awnless	C_2—Medium	D_2—Intermediate	E_1—White	F_1—Yellow	56
				F_2—Brown	11
				F_3—Black	2
				F_4—Mottled	19
			E_2—Red	F_1—Yellow	42
				F_2—Brown	12
				F_3—Black	7
				F_4—Mottled	35
			E_3—Amber	F_1—Yellow	1
		D_3—Short	E_1—White	F_1—Yellow	12
				F_3—Black	4
				F_4—Mottled	10
			E_2—Red	F_1—Yellow	7
	C_3—Fine	D_1—Long	E_1—White	F_1—Yellow	1
				F_2—Brown	2
		D_2—Intermediate	E_1—White	F_1—Yellow	6
				F_2—Brown	2
				F_3—Black	1
				F_4—Mottled	12
		D_3—Short	E_1—White	F_1—Yellow	14
				F_3—Black	7
				F_4—Mottled	6
B_2—Awned	C_1—Coarse	D_1—Long	E_1—White	F_1—Yellow	2
			E_2—Red	F_1—Yellow	1
				F_4—Mottled	2
		D_2—Intermediate	E_1—White	F_1—Yellow	33
				F_3—Black	1
				F_4—Mottled	11

Kernel				Inner glume	No. of types
	C_1—Coarse .	D_2—Intermediate .	E_2—Red .	F_1—Yellow . .	4
				F_3—Black . .	3
				F_4—Mottled . .	14
			E_3—Amber .	F_1—Yellow . .	1
		D_3—Short . .	E_1—White .	F_1—Yellow . .	4
				F_4—Mottled . .	3
	C_2—Medium .	D_1—Long . .	E_1—White .	F_1—Yellow . .	11
				F_2—Brown . .	1
				F_4—Mottled . .	5
			E_2—Red .	F_2—Brown . .	1
				F_4—Mottled . .	1
		D_2—Intermediate	E_1—White .	F_1—Yellow . .	8
				F_2—Brown . .	6
				F_4—Mottled . .	14
			E_2—Red. .	F_1—Yellow . .	22
				F_2—Brown . .	10
				F_3—Black . .	6
				F_4—Mottled . .	26
		D_3—Short .	E_1—White .	F_4—Mottled . .	3
	C_3—Fine .	D_2—Intermediate	E_1—White .	F_1—Yellow . .	7
				F_2—Brown . .	2
				F_3—Black . .	1
				F_4—Mottled . .	2
			E_2—Red . .	F_1—Yellow . .	2
		D_3—Short .	E_1—White .	F_1—Yellow . .	1
				F_2—Brown . .	3
				F_4—Mottled . .	7

Kernel				Inner glume	No. of types
		A_2—Glutinous			
B_1—Awnless	C_1—Coarse	D_1—Long	E_1—White	F_4—Mottled	3
		D_2—Intermediate	E_1—White	F_2—Brown	1
				F_4—Mottled	10
			E_2—Red	F_1—Yellow	2
				F_2—Brown	1
				F_3—Black	4
				F_4—Mottled	5
	C_2—Medium	D_1—Long	E_1—White	F_2—Brown	2
				F_4—Mottled	3
			E_2—Red	F_4—Mottled	1
		D_2—Intermediate	E_1—White	F_2—Brown	2
				F_4—Mottled	2
B_2—Awned	C_1—Coarse	D_1—Long	E_1—White	F_2—Brown	1
			E_2—Red	F_4—Mottled	2
		D_2—Intermediate	E_2—Red	F_3—Black	5
				F_4—Mottled	2
	C_2—Medium	D_1—Long	E_1—White	F_1—Yellow	9
				F_4—Mottled	1
			E_2—Red	F_4—Mottled	3
	C_3—Fine	D_1—Long	E_2—Red	F_1—Yellow	1
				Total	703

REFERENCES.

Beale, R. A. (1927). *I. I. A. R. Pusa Bull.* No. **167.**
Bhide, R. K. and Bhalerao S. G, (1927). *Mem. Dept. Agric. Ind., Bot. Ser.* **14,** No. 7.
Copeland, E. B. (1924). Rice, New York.
Crisostomo, M. (1915). *Philip. Agric. and Forester* **3.**
Graham. R. T. D. (1917). *Mem. Dept. Agric. Ind.* **6,** No. 7.
Hector, G. P. (1913). *Mem. Dept. Agric. Ind.* **6.** No. **1.**
Jacobson, H. O. (1916). *Phylippine Agric. Rev.* IX.
Joshi, K. V. and Gadkari, M. V. (1923). *Bombay. Dept. of Agric. Bull.* No. **114.**
Kikkawa, S. (1912). *J. Coll. Agric.*, Impl. Univ. Tokyo **3,** No. **2.**
Mitra, S. K., Gupta, S. N. and Ganguli, P. M. (1926). *Agric. J. Ind.* **19,** Part VI.
——— (1928). *Mem. Dept. Agric. Ind., Bot. Ser.* 15, No. 4.
Parnell, F. R.. Rangaswami Ayyangar, G. N., and Ramiah, K. (1917). *Mem. Dept. Agric. Ind.* **9** No. **2.**
Sethi, R. L. and Saxena, B. P. (1930). *Mem. Dept. Agric. Ind.* **18,** No. 6.
Thadani, K. I. and Durga Datta, H. V, (1928). *Mem. Dept. Agric. Ind.* **15.** No. 6.

APPENDIX I.

Opening of rice flowers.

(a). D. $\frac{36}{13}$ *Baurash Dumai aus.*

Date	No. of flowers opened at different times										Total	Per cent.	Weather	Remarks
	6-30 A.M.	7-30 A.M.	8-30 A.M.	9-30 A.M.	10-30 A.M.	11-30 A.M.	12-30 P.M.	1-30 P.M.	2-30 P.M.	3-30 P.M.	5-0 P.M.				
1929														
26th August	...	...	...	...	8	26	...	..	...	...	...	34	10·5	Cloudy	Average temperature—minimum 83°F, maximum 86·6°F.
27th August	...	..	1	1	50	2 3	2	..	.	...	...	77	24·0	,,	
28th August	...	...	...		46	17	4	...	...	...	...	67	20·7	,,	
29th August	...	...	...	...	...	50	17	...	...	...	...	67	20·7	Rainy morning	
30th August	...	...	...	...	...	7	28	6	...	...		41	12·7	,,	
31st August	...	...	...	...	9	12	...	...	...	...	..	21	6·5	Cloudy	
1st September	...	...	...	12	...	..	...	...	...	...	...	12	3·7	Fair	
2nd September	...	...	...	...	4	...	...	...	...	...	...	4	1·2	,,	
Total	...	...	1	13	117	135	51	6	...	...	...	323	...		
Per cent.	...	...	0·3	4·0	36·2	41·8	15·8	1·9	...	...	...	100	...		

(b). S. 483 *Babria Sail.*

Date	No. of flowers opened at different times											Total	Per cent.	Weather	Remarks
	6-30 A.M.	7-30 A.M.	8-30 A.M.	9-30 A.M.	10-30 A.M.	11-30 A.M.	12-30 P.M.	1-30 P.M.	2-30 P.M.	3-30 P.M.	5-0 P.M.				
1928															
8th October .	...	..	...	..	14	...	...	...	..	...	...	14	7·0	Fair .	Average temperature—minimum 78°F. maximum 81·1°F.
9th October .	...	...	1	..	...	1	5	10	8	4	...	29	14·5	Rainy	
10th October .	...	...	...	9	10	21	6	...	2	1	...	49	24·5	,,	
11th October .	...	1	...	1	7	22	...	...	...	...	...	31	15·5	Fair	
12th October .	..	...	1	10	18	...	..	..	...	..	...	29	14·5	,,	
13th October .	...	...	1	1	12	10	1	...	...	...	...	25	12·5	,,	
14th October .	...	...	..	...	3	6	1	...	...	.	...	10	5·0	,,	
15th October .	...	...	...	1	6	4	...	...	...	...	...	11	5·5	,,	
16th October .	...	...	...	...	2	...	...	...	...	...	...	2	1·0	,,	
Total .	...	1	3	22	72	64	13	10	10	5	...	200	...		
Per cent. .	...	·5	1·5	11·0	36·0	32·0	6·5	5·0	5·0	2·5	...	100	...		

Appendix II.

Maturity of rice.

No. of days from flowering	Av. weight of 500 grains in grms.				Av. percentage of germination			
	Broadcast *aus*	Transplanted *aus*	*Sail*	*Asra*	Broadcast *aus*	Transplanted *aus*	*Sail*	*Asra*
10	2·65	1·65	3·28	3·10	2·5	*nil*	*nil*	*nil*
12	3·80	3·10	3·95	3·65	2·5	*nil*	1·0	1·0
14	3·80	3·75	5·40	5·30	9·0	7·5	7·0	3·0
16	4·35	4·50	6·10	7·10	12·0	23·0	25·5	31·5
18	7·04	6·40	8·45	7·50	27·0	56·0	50·0	38·5
20	8·08	7·15	10·03	10·30	46·0	62·5	63·0	65·5
22	8·81	7·80	11·28	11·10	60·5	61·5	73·5	80·5
24	8·97	8·05	11·38	12·45	66·0	73·5	80·0	84·5
26	10·00	9·20	11·98	12·25	73·5	72·0	85·0	78·0
28	9·74	9·08	12·53	13·13	80·0	76·5	85·5	79·5
30	10·18	8·98	13·05	13·63	91·5	82·0	89·0	89·0
32	10·50	9·50	12·75	13·65	93·0	90·5	91·5	87·5
34	9·35	9·40	12·80	14·05	87·0	91·5	88·5	88·5
36	9·95	9·40	12·98	14·10	89·5	87·5	93·5	86·5
38	9·54	9·23	13·05	13·55	84·0	90·0	91·0	94·5
40	9·36	9·80	13·30	14·03	83·0	92·5	92·0	94·0
42	10·33	8·60	12·90	14·20	90·0	93·0	91·5	95·0
44	9·63	8·90	13·14	14·98	92·0	90·0	90·5	97·0
46	8·80	8·80	13·00	14·70	85·0	92·0	96·5	93·0
48	9·40	8·80	13·50	14·35	93·0	93·0	92·5	95·0
50	9·50	9·00	12·90	14·98	95·0	92·0	96·0	95·0

STUDIES IN INDIAN PULSES.

NO. 4. *MUNG* OR GREEN GRAM (*PHASEOLUS RADIATUS* LINN.)

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(With Plate LXIX).

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I. INTRODUCTION.

Green gram or *mung* is a very valuable pulse cultivated almost in all parts of India either as a subordinate crop with maize, sorghum, millets or cotton, or by itself as a second crop after paddy. No separate statistical records being kept for this crop, it is difficult to estimate the correct yield and area put under *mung* in India, but the aggregate outturn of this crop must be quite high as the pulse is prized very much in every part of India and is found everywhere. Almost all the seed produced is consumed in the country and very little is exported. It is one of the most nutritious of pulses, and in common with the other members of the Leguminosae, forms a necessity in the rural economy of the country. In a place like India, where a large proportion of people depends on vegetable diet alone, it is these pulse crops which furnish the proteids so necessary for life.

The following Table shows an analysis of the *mung* bean made by Yee [1920].

Protein	24·76
Sugar and starches	50·41
Cellulose	4·19
Fats	1·50
Salts	3·80
Water	11·50

Church [1886] gives the following composition of *mung* beans in 100 parts with husk :—

Composition	Type—Green seed	Type—Yellow seed	Var.—Radiatus *
Water	10·8	11·4	10·1
Albuminoids	22·2	23·8	22·7
Starch	54·0	54·8	55·8
Oil	2·7	2·0	2·2
Fibre	5·8	4·2	4·8
Ash	4·4	3·8	4·4

* *Urid* or black gram, varietal name wrongly given.

He found the nutrient ratio of the unhusked beans as 1 : 2·7, and the nutrient value to be 83.

The *mung* bean is said to be very rich in Vitamin B. It is regarded as a cure for beri-beri. In Java it is said that *mung* is consumed not so much because it is liked, as for its value as a preventive for this disease [Miguel, 1916]. In India this bean is esteemed highly, the yellow variety or *sona mung* being regarded as the most wholesome. The grain is split and generally eaten as *dal* or ground and made into balls, *bari*, *pakauri*, *papar*, etc. The green pods are sometimes taken as vegetable. As a medicinal diet, it is used in cases of flatulence and as a food in fever. The crushed stalks and leaves are much prized as fodder, and are used to give a tempting flavour to trash that even Indian cattle might otherwise reject as uneatable [Duthie and Fuller, 1882]. *Mung* like pigeon pea (*rahar*), has definitely beneficial effect on the fertility of the soil. Both these plants are deep-rooted, and therefore, introduce an additional factor, besides nitrogen fixation, that of improving the texture and content of organic matter of the deeper soil layers. Such legumes must clearly be distinguished from others like the Java indigo, which seriously deplete the supply of combined nitrogen and behave very much like cereals [Howard, 1924].

Mung is a native of India and is cultivated throughout the plains, ascending to about 6,000 feet in the outer ranges of the Northern Himalaya Mountains. Prain [1898] thinks it possible that in *Phaseolus trinervius* (an older name for which is *P. sublobatus* Roxb.) of Flora of British India we have the wild form from which perhaps both *mung* and *mash* have originated. De Candolle [1904] points out that the considerable number of varieties, and the existence of three different names in

the modern languages of India, point to a cultivation of one or two thousands years, but there is no Sanskrit name. In Africa, he says, it is probably recent. Outside India, *mung* is cultivated to some extent throughout the Southern half of Asia, in the Malayan Peninsula, in the eastern portions of East Africa and has been introduced into the Philippines, parts of America and into Greece.

Green gram—*Phaseolus radiatus* Linn., *mung, mug, pesara, kheruga, butat, ghoramuga, chhimi, pachupayaru, wuthulu*, etc.—belongs to the Natural Order *Leguminosae*, Sub-order *Papilionaceae*, tribe *Phaseoleae*. In appearance and habit of growth, *mung* resembles very closely *urid* (*P. mungo* Linn., var. *Roxburghii*) and *moth* (*P. aconitifolius* Jacq.). *Moth*, however, can be distinguished from the other two in having tri-foliate deeply lobed leaves. The resemblance of *mung* to *urid*, on the other hand, is so close that both of these are considered by some botanists as varieties of the same species. The main differences between these two pulses are brought out in the following comparison :—

TABLE I.

Showing the characteristic differences between mung *and* urid.

Character	*Mung*	*Urid*
Stem . . .	Mostly erect or sub-erect . .	Mostly spreading or trailing.
Leaves . . .	Mostly green or dark green . .	Mostly yellowish green.
Hairiness . . .	Plants clothed with hairs . .	Plants clothed with dense hairs.
Pods . . .	Spreading or reflexed, shatter readily and have short hairs.	Erect or sub-erect, do not shatter much and possess long hairs.
Seeds . . .	Small, globose, green, yellow or blackish.	Larger, oblong, green, black or dark-brown.
Seed-coat . . .	Innumerable fine wavy ridges, sometimes very faint but never lacking.	No ridges.
Hilum . . .	Not concave	Concave.

There has been considerable confusion in the nomenclature of these two species, due chiefly to Roxburgh having transposed the original Linnean names. Roxburgh names the green gram or *mung* as *Phaseolus mungo* Willd. and calls the yellow variety or *sona mung* as *P. aureus* Roxb., while *urid* is called *P. radiatus* Willd. [Roxburgh, 1832]. Duthie and Fuller [1882] also seem to have followed Roxburgh's classification. Prain has, however, reversed this nomenclature and has called *mung* as *P. radiatus* Linn. and *urid* as *P. mungo* var. *Roxburghii* Prain. He believes

that the *mung* was well known to previous botanists and well figured by Dillenius. Linneaus, though having confused it with the *urid* and even with the soybean, never gave a definite binomial name to the *mung*. Several names have been for a time adopded by botanists, which on account of confusion with other similar legumes, have made it impossible to come to a permanent agreement [Miguel, 1916]. *Phaseolus max* L., which has been referred by some botanists to the *urid*, is really the soybean, shown clearly by Linneaus' original specimen which still exists. While he intended to apply this name to the *mung*, the plant he actually described was the soybean. The name *P. mungo*, which properly applies to *tikari*, a form of *urid*, has been given to *mung*. In 1832, Roxburgh changed the application of Linneaus' names in several respects, applying the name *P. mungo* to green seeded *mung*, *P. max* to the black seeded *mung* and *P. radiatus* to the *urid*. These changes of Roxburgh cannot be accepted [Piper and Morse, 1914]. Prain's changes are, therefore, now recognized to represent the true species. These are *Phaseolus mungo* var. *Roxburghii* Prain for *urid* and *Phaseolus radiatus* Linn. for *mung*.

India is a vast country and naturally offers a wide range of soil and climatic conditions. Different varieties of the same crop are present in different localities and these are well adapted to various sets of conditions. A large variation in the habit, duration and prolificacy of the plants, as well as in the colour, size and shape of the grain, etc., has been found. No detailed classification of the different unit species commonly met with in this crop appears to have been published so far. A study of the *mung* crop was, therefore, taken up at the Botanical Section, Pusa, a few years ago. Like most other Indian field crops, *mung*, as grown by the cultivator, consists of a mixture of various types and the isolation of pure lines was therefore the first problem that needed attention. Samples from all important districts in India and Burma were collected in 1925 and single plant cultures were started from bagged seed. Isolation of pure lines was continued in the following years and 40 types in all have been evolved. It may be noted that most of the *sona mung* samples obtained from Eastern Bengal and Assam failed to do well in Pusa and were ultimately lost. It would be interesting if this variety of *mung* is studied in detail in these two provinces.

Cultivation.—*Mung* is a *kharif* crop, generally sown at the break of rains and reaped in October, but in some places it is sown in February after paddy and harvested by May. In Pusa and the neighbouring localities it is usually sown in February and not after the commencement of rains due perhaps to the possibility of heavy and continuous rain causing absolute ruin. It does best on good deep soil of fairly dense consistency and with a well distributed rainfall of 30 to 35 inches. *Mung* withstands drought well and forms a valuable food reserve when millets, etc., fail. This is an example to show why the Indian cultivator generally prefers to sow mixed

crops, which not only furnish him with all the different crops he needs for his home consumption but also saves him from utter ruin; should one crop fail, he is bound to get something from the others. The seed rate is about 12 to 15 pounds to the acre. Cultivation is the same as for cotton, millets, etc., the crop requiring a good tilth. When grown alone an average outturn of 400 to 500 lbs. of grain per acre and about three times this weight of fodder are generally obtained.

Diseases.—Mung is often susceptible to the following fungus diseases in India [Butler, 1918]:—

Powdery mildew (*Erysiphe polygoni* DC.)

Rust (*Uromyces appendiculatus* Pers. Lk.)

Root rot (*Rhizoctonia* sp.)

Leaf spot (*Cercospora cruenta* Sacc.)

Certain types not amenable to the soil and climatic conditions obtained at Pusa have suffered severely from chlorosis. A study is being made to observe the relationship of this defect with the root development of the various types.

II. General biology.

1. Flowering and pollination.

The flowers of *mung* are crowded in clusters of 10 to 20 in axillary or terminal racemes, usually the latter, and are fully self-fertile when bagged. The time of opening of the flowers is more or less the same in all types evolved and grown at Pusa. A number of buds which were likely to open on the next day were labelled in the evening and observations were made at regular hours on the next and the following days. In most cases it was found that the flowers began to open from 6 to 7 A.M. and continued to do so till about an hour later after which they remained in full bloom till about 10 or 11 A.M. Subsequently they gradually closed up and were completely closed by 2 to 4 P.M. the same evening. The next morning the corolla had a faded appearance and the least external disturbance caused it to shed. Otherwise the faded corolla generally was pushed up and carried along by the developing pod. Unlike lentils [Shaw and Bose, 1929], Indian gram [Howard, Howard and Khan, 1915] and *khesari* [Howard and Khan, 1927], in which the flowers open for two consecutive days and the pod merges out on the third or fourth day, the flowers of *mung* usually open for a single day and the developing pod can generally be seen pushing out on the very next day. On a cloudy day, however, the whole process of the opening and closing of flowers may be delayed by five or six hours, the phenomenon depending chiefly upon the temperature and humidity prevailing.

Pollination in *mung* is effected in the bud stage even before the flower opens early in the morning. Observations conducted at Pusa show that the pollen grains

are let loose from the pollen sacs between 9 and 10 P.M. in the bud stage on the night previous to the opening of the flower, and pollination is generally completed during the course of the night. This as well as the time of opening of the flowers is in agreement with the observations of Narasimham [1929] at Samalkot in Madras, who reports that till about 9 P.M. there will not be much pollination but the pollen grains will be just seen being let out of their sacs. By about 11 P.M. there will be thorough dusting of the stigma in the majority of cases and by 1-30 A.M. pollination will be complete in almost all cases. He also reports that the stigmatic hairs react to the different stages of maturity of the stigma. Till a day before the bud opens, he observes, the stigmatic hairs are invariably reflexed. By about sunset, they gradually assume a perpendicular position with reference to the style, and by 11 or 12 in the night they further incline towards the stigma and finally point towards it.

2. Cross-fertilization.

Although the structure of the floral parts of *mung* indicates its suitability for insect pollination, self-pollination is the invariable rule. No cases of natural cross-fertilization have so far been observed at Pusa. Narasimham [1929] reports the occurrence of cleistogamy, *i.e.*, fertilization taking place within unopened flowers, upto an extent of 46 per cent. and considers that self-fertilization appears to be the general rule under Godavari conditions. This is confirmed by the present studies under Pusa conditions as well. Plenty of ants, bees and some small moths, however, visit *mung* flowers very frequently and it is quite likely that natural cross-fertilization may, after all, be not absolutely impossible in this crop.

III. Important characters.

Forty types of *mung* have been isolated in the Botanical Section at Pusa from the original mixed samples collected from various parts of India. All of these are breeding true to type. The chief morphological characters in which these types vary from each other and which have been used as differentiating characters of the first magnitude, are :—

1. Seed colour,
2. Flower colour,
3. Colour of the ripe pod,

and wherever necessary further differentiation has been made in the habit of the plant.

There are differences also in the colour of the unripe pod, foliage colour, habit and time of maturity, as well as minor differences in the stem colour, but these characters have been used mostly in the description of the various types.

Duthie and Fuller [1882] describe the *mung* plant as "A hairy sub-erect annual. Stems about 2 ft. high, branching, angular. Leaves trifoliate, stipules ovate acuminate, many nerved, petioles as long or longer than the leaflets, channelled; leaflets 2-4 in., entire or more or less lobed, terminal one ovate, acute, cuneate at the base, lateral one rhomboid ovate, rounded at the base, hairy on both sides. Flowers about 6, crowded, in axillary racemes, peduncles short. Calyx about $\frac{1}{5}$ in. broad and more bi-fid above, lower portion longer and pointed. Corolla about $\frac{1}{2}$ in. long, yellow, keel beaked, spirally twisted. Stamens diadelphous. Pods 2-2$\frac{1}{2}$ in., sub-cylindrical, pointed, silky, 8-12 seeded. Seeds small, green, yellow or black".

Prain [1898] divides *mung* into three leading varieties :—

1. var. *typica*—foliage dark green, pods spreading, seeds green.
2. var. *aurea*—foliage paler, pods reflexed, seeds yellow.
3. var. *grandis*—foliage medium green, pods longer, spreading, seeds black.

In addition to these three varieties, it is proposed in the present classification to include the brown-seeded *mung* types in a fourth variety, *viz.*, var. *bruneus* Bose—foliage medium green, pods spreading, seeds brown.

The following characters of *mung* deserve more than a passing attention :—

Habit.—The general habit of a plant depends chiefly upon the height and the mode of branching. The height of the various unit species ranges from about 1 to 3 ft. The branching may be sparse or profuse. The late maturing types generally have profuse branching and are rather leafy. The *mung* plant may be erect or semi-erect, usually the latter, but some types have a spreading habit also, although they never are as trailing as *urid*. Sometimes the tips of the branches are vining.

Maturity.—The *mung* is a three month crop and commences flowering almost six weeks after sowing. At Pusa most of the types begin to flower between the sixth and the eighth week, while only a few late forms take from the ninth to the tenth week to commence their flowering. Three broad classes only—early, medium and late, have therefore been made in this classification.

Stem.—The stem is usually much branched from the base and is angular and hairy, but the hairs are not as dense as in the case of *urid*. The colour of the stem is invariably green with various degrees of purple splashes in the different types. Only Types 20 and 35 have an absolutely green stem with no purple colour on them. When the purple colour is very prominent the stem has been described as purplish green, example Type 16; but when these splashes on the stem are fewer the stem has been called green with purplish splashes, example Type 1, or green with few purplish splashes as in Type 2.

Leaves.—The leaves of *mung* are trifoliate with rather large, ovate, entire or rarely lobed leaflets. The leaflets are membranous with scattered adpressed hairs on both sides and are 2 to 4 in. long. The terminal leaflet is ovate, acute, cuneate at the base, while the lateral ones are rhomboid ovate, and rounded at the base. Three grades, *viz.*, large, medium and small have been made in the size of the leaves in the description of types which is to follow. The colour of the foliage is dark green, green or light green, but never yellowish green like *urid.* The petiole is channelled and as long as, or even longer than, the leaflets. In most cases the petiole is green with distinct purplish patches on it, and only in Types 20 and 35 is it absolutely green.

Flowers.—There is only a very slight difference in the size of the flower in *mung*, the width of the standard usually ranging from 12 to 16 mm. only. This character has not therefore been studied in any detail. Definite variation, however, exists in the colour of the flower, and four distinct grades have been recognized.

1. Flowers with reed-yellow standard, wing and keel. Example Types 20 and 35.
2. Flowers with olive-yellow standard, strontium-yellow wings and yellowish olive keel. Example Types 4, 5, etc.
3. Flowers with yellowish-olive standard, yellowish-citrine wings and yellowish-olive keel. Example Types 28, 29, etc.
4. Flowers with light yellowish-olive standard, strontium-yellow wings and yellowish-olive keel. Example Types 1, 2, etc.

Ridgway's [1912] colour standards have been used to determine the various tints described above. Colour examination should always be done *en masse*, *i.e.*, by taking 20 or more flowers at a time from a pure breeding type and considering the average colour of each part of the flower. Individual flowers from the same plant may show slight variation in their tints due to the influence of so many external factors. After seed colour the colour of the flower has been taken as a character of next importance in separating out the various types from each other. The colour of the standard has always been taken to represent the flower colour of each type.

Pods.—The pods of *mung* are generally clustered round a terminal or axillary rachis and are slender and long, being about 2 to 3 in. in length. They are sub-cylindrical and somewhat hairy. They may be straight or slightly curved, but often it becomes difficult to recognize this character, as the same plant may show pods of both shape, although there generally is a predominance of one kind. Each pod contains 10 to 15 seeds. The colour of the ripe pod has also been taken as a differentiating character in the present studies. There is some variation also in the colour of the unripe pods.

The following grades of colour have been recognized :—

Colour of the ripe pod.

1. Iron-grey. Example Type 28.
2. Olive-grey. Example Type 3.
3. Snuff-brown. Example Type 5.
4. Light brown. Example Type 9.

Colour of the unripe pod.

1-*a*. Dark green. Example Type 13.

1-*b*. Dark green but with distinct red veins on the ventral suture. Example Type 16.

2-*a*. Green. Example Type 7.

2-*b*. Green with distinct red veins on the ventral suture. Example Type 1.

3-*a*. Light green. Example Type 34.

3-*b*. Light green with distinct red veins on the ventral suture. Example Type 11.

Seed.—This has furnished the most important character for the classification of the various types of *mung* and has been considered in the present study as the main differentiating character. The seeds are globose or oblong, green in most varieties, but in others they may be yellow, brown or marbled with black patches. In addition to this the different coloured seeds may be either shining or dull. The following grades of seed colour have been included in the present study.

Brown—

(1) Dark brown or clove-brown with distinct ridges or corrugations on the seed coat. Example Types 1 to 4.

(2) Light brown with distinct corrugations on the seed coat. Example Type 5.

Blackish—

(1) Ground colour dark green with blackish marbling.

(*a*) Grains shining. Example Types 6 and 7.

(*b*) Grains dull. Example Type 8.

(2) Ground colour green with blackish marbling.

(*a*) Grains shining. Example Type 9.

Dark green—

(*a*) Grains shining. Example Types 10 to 15.

(*b*) Grains dull. Example Types 16 to 20.

Green—

(*a*) Grains shining. Example Types 21 to 27.

(*b*) Grains dull. Example Types 28 to 35.

Yellow--

(1) Pale lemon-yellow with shining grains. Example Type 36.

(2) Greenish yellow.

(*a*) Grains shining. Example Type 37.

(*b*) Grains dull. Example Types 38 to 40.

The seed coat in *mung* is marked by innumerable fine wavy ridges or corrugations which are sometimes very prominent as in Types 1 to 5, or are generally very faint and not easily recognizable with the naked eye, but they are apparently never lacking. This is another point of distinction between the seeds of *mung* and *urid*. Sometimes nearly smooth seeds may be found in the same pod with others strongly striate. The hilum in *mung*, unlike that of *urid*, is not concave.

IV. Key to the types of *mung* (*Phaseolus radiatus* Linn.).

	Old culture No.	Type No.
Variety Bruneus (*Bose*).		
I. Seeds brown		
(1) Dark brown or clove-brown		
Flowers light yellowish olive		
Pods iron-grey	M. 80	1
Pods snuff-brown	S. 2	2
Flowers olive-yellow		
Pods olive-grey	M. 79-1	3
Pods snuff-brown	S. 6	4
(2) Light brown		
Flowers olive-yellow		
Pods snuff-brown	M. 44	5
Variety Grandis (*Prain*).		
II. Seeds green with blackish marbling		
(1) Ground colour dark green		
(*a*) Grains shining		
Flowers olive-yellow		
Pods snuff-brown		
Habit semi-erect		
Leaves large	M. 49	6
Leaves medium in size	M. 3	7

PLATE LXIX.

FLOWER COLOUR.

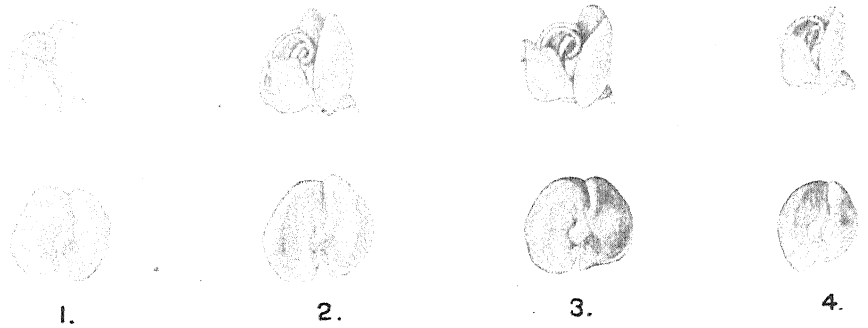
Fig. 1.—Standard colour—*Reed-yellow.* Type 20.
Fig. 2.—Standard colour—*Olive-yellow.* Type 4.
Fig. 3.—Standard colour—*Yellowish olive.* Type 28.
Fig. 4.—Standard colour—*Light yellowish olive.* Type 1.

POD COLOUR.

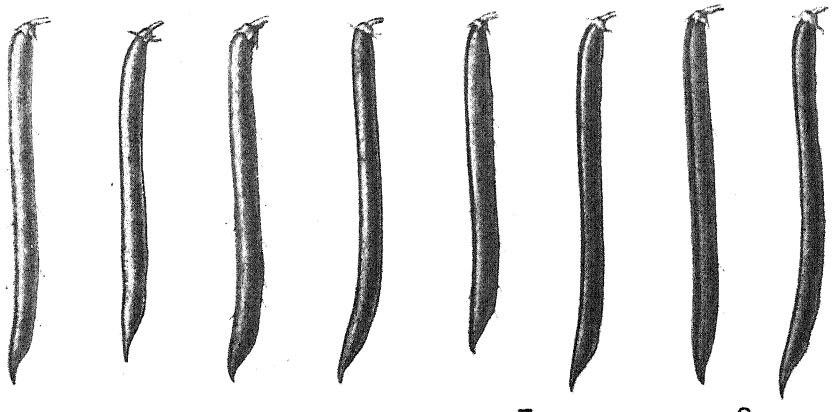
Fig. 5.—Unripe pod—*Light green.* Ripe pod—*Light brown.* Type 9.
Fig. 6.—Unripe pod—*Green.* Ripe pod—*Snuff brown.* Type 4.
Fig. 7.—Unripe pod—*Dark green with distinct vein on suture.* Ripe pod—*Olive-grey.* Type 16.
Fig. 8.—Unripe pod—*Dark green with distinct vein on suture.* Ripe pod—*Iron-grey* Type 17.

SEED COLOUR.

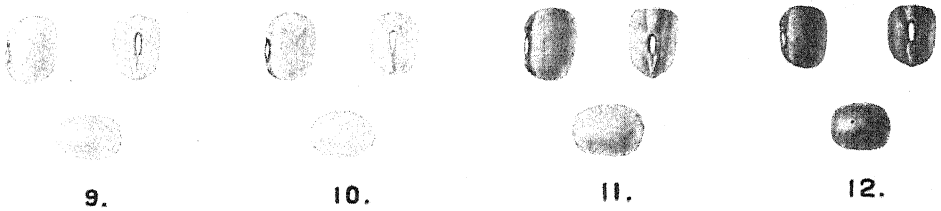
Fig. 9.—Seeds—*Pale lemon-yellow, shining.* Type 36.
Fig. 10.—Seeds—*Greenish yellow, shining.* Type 37.
Fig. 11.—Seeds—*Greenish yellow, dull.* Type 38.
Fig. 12.—Seeds—*Green and dull.* Type 35.
Fig. 13.—Seeds—*Green and shining.* Type 22.
Fig. 14.—Seeds—*Dark green and dull.* Type 18.
Fig. 15.—Seeds—*Dark green and shining.* Type 10.
Fig. 16.—Seeds—*Light brown.* Type 5.
Fig. 17.—Seeds—*Dark brown.* Type 4.
Fig. 18.—Seeds—*Dark green with blackish marbling dull.* Type 8.
Fig. 19.—Seeds—*Green with blackish marbling, shining.* Type 9.
Fig. 20.—Seeds—*Dark green with blackish marbling, shining.* Type 7.



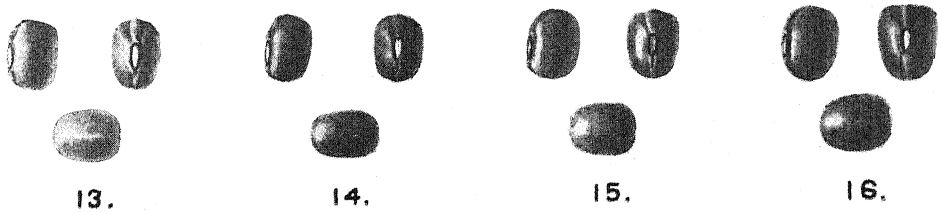
1. 2. 3. 4.



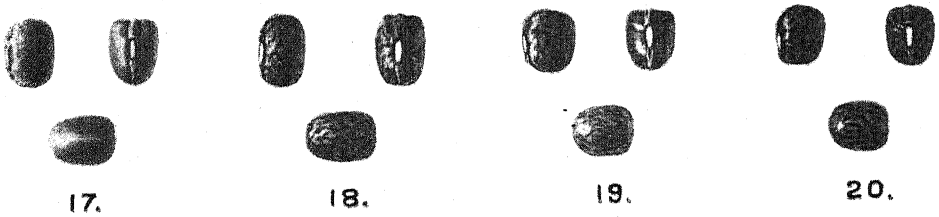
5. 6. 7. 8.



9. 10. 11. 12.

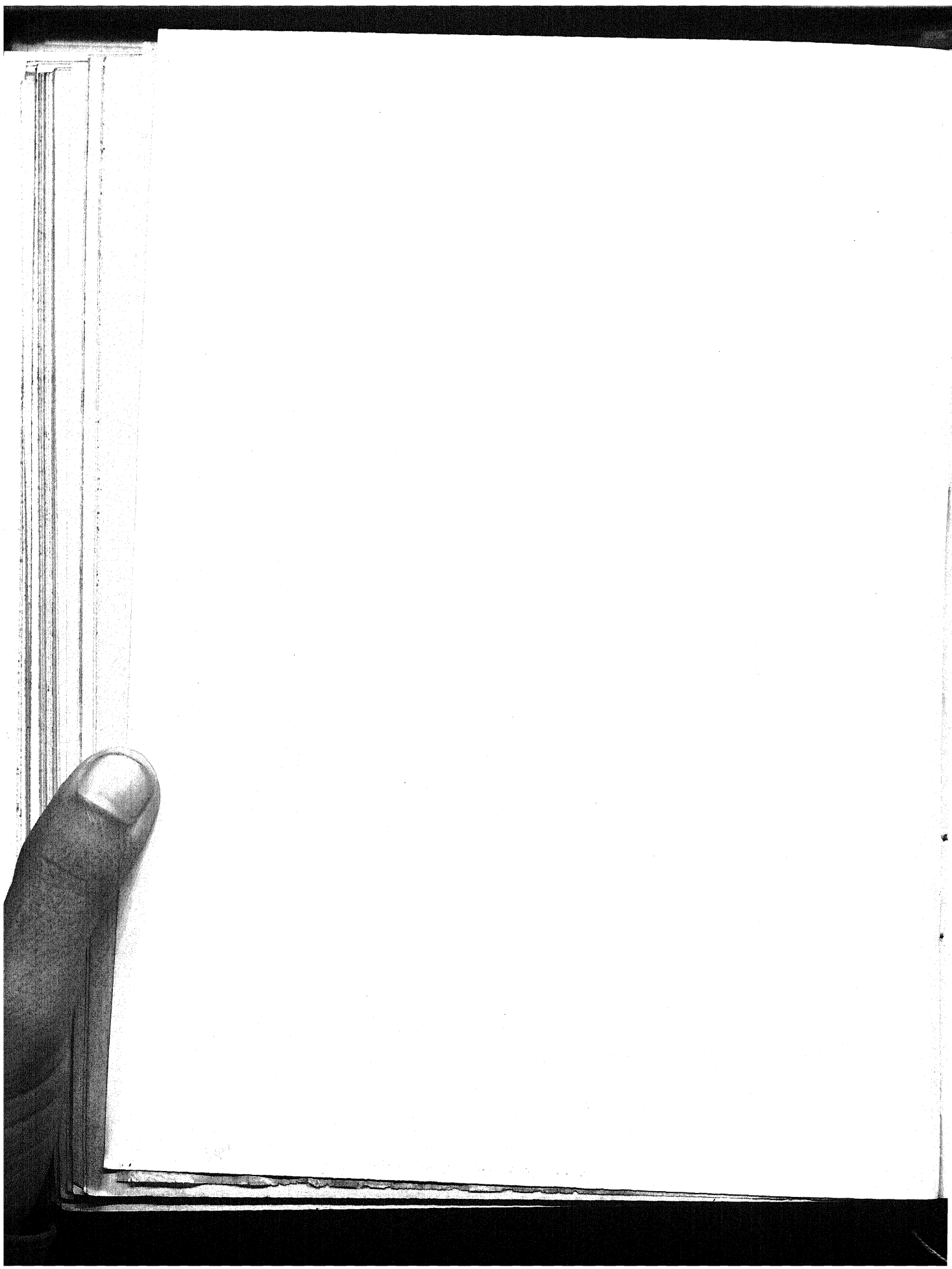


13. 14. 15. 16.



17. 18. 19. 20.

Flower, pod and seed colour in *mung*.



	Old culture No.	Type No.
Variety Grandis (*Prain*)—contd.		
II. Seeds green with blackish marbling—contd.		
(1) Ground colour dark green—*contd.*		
(*b*) Grains dull		
Flowers olive-yellow		
Pods snuff-brown	M. 22-2	8
(2) Ground colour green		
(*a*) Grains shining		
Flowers olive-yellow		
Pods light brown	S. 3	9
Variety Typica (*Prain*).		
III. Seeds dark green		
(*a*) Grains shining		
Flowers light yellowish olive		
Pods olive-grey		
Habit semi-erect	M. 29	10
Habit spreading	M. 25	11
Flowers olive-yellow		
Pods olive-grey		
Habit semi-erect	M. 50	12
Habit spreading	S. 4	13
Pods snuff-brown	M. 33	14
Pods light brown	M. 48	15
(*b*) Grains dull		
Flowers yellowish olive		
Pods olive-grey	M. 60	16
Flowers light yellowish olive		
Pods iron-grey	M. 65	17
Pods olive-grey	M. 75	18
Pods snuff-brown	M. 46	19
Flowers reed-yellow		
Pods iron-grey	M. 63	20

	Old culture No.	Type No.
Variety Typica (*Prain*)—contd.		
IV. Seeds green		
(*a*) Grains shining		
Flowers light yellowish olive		
Pods olive-grey	M. 28-2	21
Pods snuff-brown	M. 45	22
Pods light brown	M. 74	23
Flowers olive-yellow		
Pods olive-grey	M. 70	24
Pods snuff-brown		
Habit semi-erect	M. 28	25
Habit spreading	M. 31	26
Pods light brown	M. 73	27
(*b*) Grains dull		
Flowers yellowish olive		
Pods iron-grey		
Habit semi-erect	M. 53	28
Habit spreading	M. 51-1	29
Flowers light yellowish olive		
Pods iron-grey	M. 58	30
Pods olive-grey	M. 57	31
Pods light brown	M. 77	32
Flowers olive-yellow		
Pods olive-grey		
Habit semi-erect	M. 69	33
Habit spreading	M. 41	34
Flowers reed-yellow		
Pods iron-grey	M. 42	35

	Old culture No.	Type No.
Variety Aureus (*Prain*).		
V. Seeds yellow		
(1) Pale lemon-yellow		
(*a*) Grains shining		
Flowers olive-yellow		
Pods olive-grey	M. 11	36
(2) Greenish yellow		
(*a*) Grains shining		
Flowers olive-yellow		
Pods olive-brown	M. 21-1	37
(*b*) Grains dull		
Flowers olive-yellow		
Pods olive-grey		
Habit semi-erect	M. 21-2	38
Habit spreading	*Brahmanbaria*	39
Flowers light yellowish olive		
Pods olive-grey	M. 20	40

V. Description of *mung* types, *Phaseolus radiatus* (Linn.).

Variety—Bruneus (*Bose*).

Type 1.—Early, spreading, robust growth. *Stem* green with purplish splashes. *Leaves* medium in size, foliage green. *Flowers* light yellowish olive. *Inflorescence* both axillary and terminal. *Pods*—Unripe green with red vein on the suture ; ripe iron-grey. *Seeds* dark brown with distinct corrugations.

Type 2.—Early, spreading, very leafy and robust growth. *Stem* green with few purplish splashes. *Leaves* medium in size, foliage light green. *Flowers* light yellowish olive. *Inflorescence* both axillary and terminal. *Pods*—Unripe green with red veins on the suture ; ripe snuff-brown. *Seeds* dark brown with distinct corrugations.

Type 3.—Early, spreading, rather leafy. *Stem* green with purplish splashes. *Leaves* medium in size, foliage green. *Flowers* olive-yellow. *Inflorescence* both

axillary and terminal. *Pods*—Unripe green ; ripe olive-grey. *Seeds* dark brown with distinct corrugations.

Type 4.—Early, spreading, good growth. *Stem* green with purplish splashes. *Leaves* medium in size, foliage green. *Flowers* olive-yellow. *Inflorescence* both axillary and terminal. *Pods*—Unripe green ; ripe snuff-brown. *Seeds* dark brown with distinct corrugations.

Type 5.—Early, semi-erect, fairly bushy. *Stem* green with few purplish splashes. *Leaves* medium in size, foliage green. *Flowers* olive-yellow. *Inflorescence* both axillary and terminal. *Pods*—Unripe green ; ripe snuff-brown. *Seeds* light brown with distinct corrugations.

Variety—Grandis (*Prain*).

Type 6.—Medium early in maturity, semi-erect and bushy, very leafy but a poor yielder. *Stem* green with purplish splashes. *Leaves* large in size, foliage green. *Flowers* olive-yellow. *Inflorescence* both axillary and terminal. *Pods*—Unripe green with red veins on the suture ; ripe snuff-brown. *Seeds* very dark green with blackish marbling, almost black, grains shining.

Type 7.—Early, semi-erect and bushy, very leafy. *Stem* green with purplish splashes. *Leaves* medium in size, foliage green. *Flowers* olive-yellow. *Inflorescence* both axillary and terminal. *Pods*—Unripe green, ripe snuff-brown. *Seeds* dark green with blackish marbling, grains shining.

Type 8.—Early, semi-erect, good growth. *Stem* green with purplish splashes. *Leaves* medium in size, foliage green. *Flowers* olive-yellow. *Inflorescence* mostly terminal. *Pods*—Unripe green with red veins on the suture ; ripe snuff-brown. *Seeds* dark green with blackish marbling, grains dull.

Type 9.—Early, semi-erect, leafy and robust. *Stem* green with purplish splashes. *Leaves* medium in size, foliage green. *Flowers* olive-yellow. *Inflorescence* both axillary and terminal. *Pods*—Unripe green ; ripe light brown. *Seeds* green with blackish marbling, grains shining.

Variety—Typica (*Prain*).

Type 10.—Medium early in maturity, semi-erect, heavy yielder. *Stem* green with few purplish splashes. *Leaves* medium in size, foliage green. *Flowers* light yellowish olive. *Inflorescence* mostly terminal. *Pods*—Unripe light green with very distinct red veins on the suture ; ripe olive-grey. *Seeds* dark green, shining.

Type 11.—Early, spreading, poor growth. *Stem* green with few purplish splashes. *Leaves* medium in size, foliage green. *Flowers* light yellowish olive. *Inflorescence* both axillary and terminal. *Pods*—Unripe light green with red veins on the suture ; ripe iron-grey. *Seeds* dark green, shining.

Type 12.—Early, semi-erect and bushy, rather leafy. *Stem* green with few purplish splashes. *Leaves* medium in size, foliage green. *Flowers* olive-yellow. *Inflorescence* both axillary and terminal. *Pods*—Unripe green ; ripe iron-grey. *Seeds* dark green, shining.

Type 13.—Early, spreading, robust growth. *Stem* green with few purplish splashes. *Leaves* medium in size, foliage green. *Flowers* olive-yellow. *Inflorescence* both axillary and terminal. *Pods*—Unripe dark green ; ripe olive-grey. *Seeds* dark green, shining.

Type 14.—Early, semi-erect, poor growth. *Stem* green with purplish splashes. *Leaves* medium in size, foliage light green. *Flowers* olive-yellow. *Inflorescence* both axillary and terminal. *Pods*—Unripe green ; ripe snuff-brown. *Seeds* dark green, shining.

Type 15.—Early, semi-erect rather leafy. *Stem* green with purplish splashes. *Leaves* medium in size, foliage green. *Flowers* olive-yellow. *Inflorescence* both axillary and terminal. *Pods*—Unripe green ; ripe light brown. *Seeds* dark green, shining.

Type 16.—Medium early in maturity, semi-erect, prolific. *Stem* purplish green. *Leaves* medium in size, foliage green. *Flowers* yellowish olive. *Inflorescence* mostly terminal. *Pods*—Unripe dark green with red veins on the suture ; ripe olive-grey. *Seeds* dark green, dull.

Type 17.—Late in maturity, erect and bushy, plants rather tall. *Stem* green with a few purplish splashes. *Leaves* large, foliage dark green. *Flowers* light yellowish olive. *Inflorescence* both axillary and terminal. *Pods*—Unripe dark green with red veins on the suture ; ripe iron-grey. *Seeds* dark green, dull.

Type 18.—Early, semi-erect, prolific. *Stem* purplish green. *Leaves* medium in size, foliage green. *Flowers* light yellowish olive. *Inflorescence* mostly terminal. *Pods*—Unripe green with red veins on the suture ; ripe olive-grey. *Seeds* dark green, dull.

Type 19.—Medium in maturity, spreading, bushy and prolific. *Stem* green with purplish splashes. *Leaves* medium in size, foliage green. *Flowers* light yellowish olive. *Inflorescence* both axillary and terminal. *Pods*—Unripe green with red veins on the suture ; ripe snuff-brown. *Seeds* dark green, dull.

Type 20.—Medium early in maturity, semi-erect. *Stem* green. *Leaves* medium in size, foliage green. *Flowers* reed-yellow. *Inflorescence* both axillary and terminal. *Pods*—Unripe green ; ripe iron-grey. *Seeds* dark green, dull.

Type 21.—Early, spreading, rather prolific. *Stem* green with a few purplish splashes. *Leaves* medium in size, foliage light green. *Flowers* light yellowish olive. *Inflorescence* mostly terminal. *Pods*—Unripe green ; ripe olive-grey. *Seeds* green, shining.

Type 22.—Early, spreading, prolific. *Stem* green with purplish splashes. *Leaves* medium in size, foliage green. *Flowers* light yellowish olive. *Inflorescence* both axillary and terminal. *Pods*—Unripe light green with red veins on the suture ; ripe snuff-brown. *Seeds* green, shining (seed colour somewhat lighter than that in other types).

Type 23.—Early, semi-erect, good setting. *Stem* green with purplish splashes. *Leaves* large, foliage green. *Flowers* light yellowish olive. *Inflorescence* both axillary and terminal. *Pods*—Unripe green with red veins on the suture ; ripe light brown. *Seeds* green, shining.

Type 24.—Early, semi-erect, poor growth but prolific. *Stem* green with purplish splashes. *Leaves* medium in size, foliage light green. *Flowers* olive-yellow. *Inflorescence* mostly terminal. *Pods*—Unripe green ; ripe olive-grey. *Seeds* green, shining.

Type 25.—Early, semi-erect, bushy and prolific. *Stem* green with few purplish splashes. *Leaves* large, foliage light green. *Flowers* olive-yellow. *Inflorescence* both axillary and terminal. *Pods*—Unripe green ; ripe snuff-brown. *Seeds* green, shining.

Type 26.—Early, spreading. *Stem* green with purplish splashes. *Leaves* medium in size, foliage green. *Flowers* olive-yellow. *Inflorescence* both axillary and terminal. *Pods*—Unripe light green with red veins on the suture ; ripe snuff-brown. *Seeds* green, shining.

Type 27.—Early, semi-erect and bushy, rather leafy. *Stem* green with purplish splashes. *Leaves* large, foliage green. *Flowers* olive-yellow. *Inflorescence* both axillary and terminal. *Pods*—Unripe green with red veins on the suture ; ripe light brown. *Seeds* green, shining.

Type 28.—Early, semi-erect, very prolific. *Stem* green with purplish splashes. *Leaves* medium in size, foliage green. *Flowers* yellowish olive. *Inflorescence* mostly terminal. *Pods*—Unripe dark green with red veins on the suture ; ripe iron-grey. *Seeds* green, dull.

Type 29.—Early, spreading and rather bushy. *Stem* green with purplish splashes. *Leaves* medium in size, foliage green. *Flowers* yellowish olive. *Inflorescence* mostly terminal. *Pods*—Unripe green with red veins on the suture ; ripe iron-grey. *Seeds* green, dull.

Type 30.—Late, spreading, bushy and prolific. *Stem* green with purplish splashes. *Leaves* large, foliage dark green. *Flowers* light yellowish olive. *Inflorescence* mostly terminal. *Pods*—Unripe green with red veins on the suture ; ripe iron-grey. *Seeds* green, dull.

Type 31.—Late, spreading, very prolific. *Stem* green with purplish splashes. *Leaves* medium in size, foliage green. *Flowers* light yellowish olive. *Inflorescence*

both axillary and terminal. *Pods*—Unripe green with red veins on the suture ; ripe olive-grey. *Seeds* green, dull.

Type 32.—Late, spreading. *Stem* green with purplish splashes. *Leaves* small in size, foliage green. *Flowers* light yellowish olive. *Inflorescence* both axillary and terminal. *Pods*—Unripe green with red veins ; ripe light brown. *Seeds* green, dull.

Type 33.—Early, semi-erect, poor growth. *Stem* green with purplish splashes. *Leaves* medium in size, foliage green. *Flowers* olive-yellow. *Inflorescence* both axillary and terminal. *Pods*—Unripe green ; ripe olive-grey. *Seeds* green, dull.

Type 34.—Early, spreading, very leafy but a poor yielder. *Stem* green with purplish splashes. *Leaves* small in size, foliage green. *Flowers* olive-yellow. *Inflorescence* both axillary and terminal. *Pods*—Unripe light green ; ripe olive-grey. *Seeds* green, dull.

Type 35.—Medium early in maturity, semi-erect. *Stem* green. *Leaves* medium in size, foliage light green. *Flowers* reed-yellow. *Inflorescence* both axillary and terminal. *Pods*—Unripe green ; ripe iron-grey. *Seeds* green, dull.

Variety—Aureus (*Prain*).

Type 36.—Early, semi-erect. *Stem* green with purplish splashes. *Leaves* medium in size, foliage light green. *Flowers* olive-yellow. *Inflorescence* both axillary and terminal. *Pods*—Unripe green ; ripe olive-grey. *Seeds* pale lemon-yellow, shining.

Type 37.—Medium early in maturity, semi-erect. *Stem* green with a few purplish splashes. *Leaves* medium in size, foliage light green. *Flowers* olive-yellow. *Inflorescence* mostly terminal. *Pods*—Unripe green with red veins on the suture ; ripe olive-grey. *Seeds* greenish yellow, somewhat shining.

Type 38.—Medium early in maturity, semi-erect. *Stem* green with a few purplish splashes. *Leaves* medium in size, foliage light green. *Flowers* olive-yellow. *Inflorescence* both axillary and terminal. *Pods*—Unripe green with red veins on the suture ; ripe olive-grey. *Seeds* greenish yellow, dull.

Type 39.—Late in maturity, spreading, bushy, rather leafy. *Stem* green with a few purplish splashes. *Leaves* small in size, foliage green. *Flowers* olive-yellow. *Inflorescence* both axillary and terminal. *Pods*—Unripe green ; ripe olive-grey. *Seeds* greenish yellow, dull.

Type 40.—Medium early in maturity, semi-erect, leaves rather sparse. *Stem* green with purplish splashes. *Leaves* medium in size, foliage light green. *Flowers* light yellowish olive. *Inflorescence* both axillary and terminal. *Pods*—Unripe green with red veins on the suture ; ripe olive-grey. *Seeds* greenish yellow, dull.

VI. REFERENCES.

Bowman, H. H. M., and Yee, M. A. (1927). Papers Michigan Acad. Sci. Arts and Letters, **6**, 181.

Butler, E. J. (1918). Fungi and Disease in Plants, 256-262.

Church, A. H. (1886). Food Grains of India, 151.

De Candolle, A. (1904). Origin of Cultivated Plants, London, 346.

Duthie, J. F., and Fuller, J. B. (1882). Field and Garden Crops of the N.-W. Prov. & Oudh, **1**, 37-40.

Howard, A. (1924). Crop Production in India, Oxford Univ. Press.

Howard, G. L. C., and Khan, A. R. (1924). *Mem. Dept. Agric. Ind., Bot. Ser.* 12, No. 4.

———(1927). *Ibid* **15**, No. 2.

Miguel, L. A. S. (1916). *Philippine Agric. and Forester* **5**, 167.

Narasimham, M. A. (1929). *Agric. J. Ind.* **24**, 397-401.

Piper, C. V., and Morse, W. J. (1914). *U. S. Dept. Agric. Bull.* No. 119.

Prain, D. (1898). *J. Asiatic Soc. of Bengal*, **66**, 422.

——— (1903). Bengal Plants, 1, 387-388.

Ridgway, R. (1912). Colour Standards and Colour Nomenclature Washington, D. C.

Roxburgh, W. (1832). Flora Indica, **3**, 292.

Shaw, F. J. F., and Bose, R. D. (1928). *Mem. Dept. Agric. Ind., Bot. Ser.* **16**, No. 6.

STUDIES IN INDIAN PULSES.

NO. 5. *URID* OR BLACK GRAM (*PHASEOLUS MUNGO* LINN. VAR. *ROXBURGHII* PRAIN).

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(Received for publication on the 11th July 1932)

(With Plate LXX).

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I. INTRODUCTION.

Urid or black gram is the most highly prized of the pulses of the genus *Phaseolus*, and is cultivated in almost all parts of India. Like *mung* [Bose, 1932] no separate statistics of acreage or production of this pulse is maintained, but the yield and area under this crop must be very considerable as it enters largely into the vegetarian diet of the high caste Hindus. Its economic value is great though the volume of exports is negligible. Flavoured with aromatics and condiments, it is largely utilized in internal consumption. Sometimes it is even grown for green-manuring.

Church [1886] gives the following chemical composition in 100 parts of the unhusked grain :—

Water	10·1	
Albuminoids	22·7	
Starch	55·8	
Oil	2·2	
Fibre	4·8	
Ash	4·4	(Of which 1·1 consists of phosphoric acid).

Leather [1903] gives the following analyses for *mung* and *urid*, but it may be remembered that his nomenclature of the two pulses is wrong. *P. mungo* according to him was *mung* and *P. mungo* var. *radiatus* was *urid* :—

	P. mungo (*mung*)		*P. mungo* var. *radiatus* (*urid*)	
	Average of six samples	Leaves and husks from threshing floor	Average of five samples	Leaves and husks from threshing floor
Water	9·97	15·38	10·38	13·30
Ash	4·57	14·92	4·12	12·29
Cellulose	3·81	17·08	3·80	18·66
Fat	0·93	1·70	1·07	2·52
Non-nitrogenous matter	58·29	38·24	56·76	39·67
Nitrogenous matter	23·43	12·68	23·87	11·56
Nitrogen	3·59	2·03	3·82	1·85
Nitrogen as protein	3·33	1·79	3·40	1·74

It resembles *mung* (*Phaseolus radiatus* Linn.) very closely in all its constituents.

Like *mung* it is boiled and eaten whole or after being split, in the form of *dal*. Parched and ground to flour, it is made into balls with spice (*pakauri daibarra*, etc.) or it is eaten in the form of a sort of porridge or baked into bread—it is the chief constituent of the wafer biscuit known as *papar*. *Bari* and *sepa* are two other preparations commonly made out of *urid*. The green pods are eaten as a vegetable [Watt, 1908]. In Hindu medicine it is highly valued, and is prescribed both internally and externally in paralysis, rheumatism and affections of the nervous system. It is considered as easily digestible and as a cooling article of diet [Murray, 1892]. The seed is the reputed origin of the weight known as *masha*, twelve of which go to the *tola*, and 960 to the *seer* (2 lb.).

The *urid* is a native of India and the presence of a Sanskrit name *mash* suggests that the crop has been known in this country from very old times. Prain [1898] considers *Phaseolus trinervius* or *P. sublobatus* Roxb. as the wild form from which perhaps both *mash* and *mung* have originated. During modern times *urid* has been introduced into many tropical parts of both the old and the new worlds. Some of the late varieties make a dense mass of herbage and one of these is used as a green-manure crop in the West Indies under the name of Woolly Pyrol [Piper, 1915].

Black gram—*Phaseolus mungo*, var. *roxburghii*, Prain, *urid* (or *urd*), *dord*, *tircorat-kulai*, *mash-kulai*, *ramra udid*, *adad*, *ulunda*, *minu-mulu*, *hasaru*, etc., belongs to the Natural Order *Leguminosae*; sub-order *Papilionaceae* tribe *Phaseoleae*. In appearance and growth it resembles *mung* (*P. radiatus* Linn.) very closely, so much so that some botanists consider both of these as varieties of the same species. The main differences between these two pulses have been shown in the previous publication [Bose, 1932].

Cultivation.—*Urid* has two distinct cultivated forms, one with large black seeds, which ripens in August and September and which is generally called *urid* or *urd*, and the other with small green seeds, which ripens in October or November and which sometimes is given the diminutive name of *urdi*. Both are generally sown at the commencement of the rains and need the same cultural and soil treatments. It is thus a *kharif* crop although occasionally it is also sown, like *mung*, in February and reaped in May. It prefers the heavier classes of soils. In Northern Bihar, however, it is generally sown mixed with maize in lighter soils as well. Two or three ploughings are enough for this crop. Too much cultivation and a fine tilth are apt to encourage excessive foliage growth at the cost of the yield of grain. The seed rate is about 10 to 12 pounds to the acre. When grown alone the average outturn of *urid* is about 400 lb. of grain to the acre, with about three times this weight of straw. Both the grain and the straw are useful as horse and cattle food, although the hairy nature of the stem and leaves prevents its being relished much by these animals.

Notwithstanding the importance and the variety of interesting problems they offer for investigation, the pulse crops in India have not received the attention they deserve. Although a large variation in the habit, duration and morphological characters of the *urid* plants exists, no detailed classification of the different unit species commonly met with in this crop appears to have been published so far in India. Samples of *urid* from all important districts of India and Burma were, therefore, collected in 1925 and single plant cultures from this were started from selfed plants. Isolation of pure lines was continued during the following years and it has been possible to separate out 25 types from this A large number of forms, however, including some very bold-seeded varieties from the Central Provinces did not adapt themselves to the soil and climatic conditions of Pusa and were gradually eliminated during the course of the work. Some types have suffered severely from chlorosis and the relation of this with the root development and other morphological characters deserves further study. Ghosh and Basu [1925] studied the effect of lead carbonate, zinc sulphate, manganese sulphate, magnesium sulphate, lime, gypsum, boric acid, potassium iodide and sodium chloride in improving the condition of the crop and preventing chlorosis, but they could not control the yellowing

of the leaves to any great extent. In all the plots, however, to which sulphates were applied the growth of the healthy plants was distinctly more vigorous, gypsum giving the most favourable results.

Pests and Diseases.—Besides chlorosis or yellowing of the leaves *urid* is often susceptible to the following fungus diseases in India [Butler, 1918]:—

Powdery mildew (*Erysiphe polygoni*, DC).

Rust (*Uromyces appendiculatus* Pers. Lk.).

Leaf spot (*Cercospora cruenta* Sacc.).

A lot of damage is sometimes done to this crop, in Northern Bihar, by the Bihar hairy caterpillar (*Diacrisia obliqua* Wlk.), swarms of which feed on the leaves, and the only way to avoid them is to be on the look out for their egg masses or the newly hatched young ones and picking them out before much harm is done to the crop.

II. General biology.

1. Flowering and pollination.

The flowers of *urid* are borne in capitate clusters of 5-6 on the end of stout, hairy, peduncles and are fully self-fertile. The time of opening of flowers is more or less the same in all types evolved and grown at Pusa and resembles that of *mung* almost in all respects [Bose, 1932]. The flowers generally begin to open early in the morning, from 6 to 7 A.M. and continue to do so till about 9 A.M. after which they remain fully open till about noon, when they gradually close up and are completely so till about 2 to 4 P.M. The next morning the faded corolla may be seen being pushed up and carried along by the developing pod.

Pollination in *urid* as in *mung* is effected in the bud stage the night previous to the opening of the flowers. Narasimham [1929] reports that till about 9 P.M. there will not be much pollination but the pollen grains will be just seen being let out of their sacs. By about 11 P.M. there will be a thorough dusting of the stigma in the majority of cases and by 1-30 A.M. pollination will be complete in almost all cases. This is in close agreement to the observations made at Pusa.

2. Cross-fertilization.

Self-pollination is the general rule in this crop and no case of natural cross-fertilization has so far been observed at Pusa. The various types have been grown close to each other for nearly six years but no natural cross has been detected yet. Narasimham [1929] observed cleistogamy or fertilization within unopened flowers to an extent of 42 per cent. and considers that self-fertilization is the general rule in *urid*.

III. IMPORTANT CHARACTERS.

Twenty-five types of *urid* have been evolved in the Botanical Section at Pusa, from the original mixed samples collected from all important districts of India and Burma. All of these are breeding true to type and represent more or less the main types cultivated in this country. Seed colour has already an established usage in commercial identification, and its employment as the primary basis of classification is both expedient and constructive. Seed, flower and pod colour are the principal bases of division in the present classification.

There are differences also in the foliage colour, habit, time of maturity as well as minor differences in the colour and hairiness of the stem, pods, etc. All these characters have been used in the description of the types which is to follow.

In botanical characters *urid* resembles *mung* very closely as reported previously [Bose, 1932], but in general behaviour the plants of the former are lower and spreading or rather trailing on the ground, while those of the latter are mostly erect or semi-erect. The plant may be described as follows :—

A spreading annual, usually with procumbent branches. Stems diffuse, furrowed and densely clothed with long brown hairs ; the extent of hairiness differing in different types. Leaves large, trifoliate, hairy, stipules ovate, acuminate ; petioles very long and hairy, generally having diffused purple colour. Leaflets very broadly ovate or nearly rhomboid, orbicular, usually entire, thin, short, acute ; foliage colour mostly light green, but green or even dark green in a few types. It is usually lighter than that of *mung*. Flowers fully self-fertile, lemon or pale yellow, in capitate clusters of five or six on the end of rather stout hairy peduncles. Calyx connate in a companulate tube. Corolla pale or lemon-yellow, standard orbicular, subauriculate at base, wings ovate or oblong, adnate to the keel, keel prolonged in a beak to form a spiral. Stamens diadelphous. Pods erect or suberect, about 2 in. long nearly cylindrical, somewhat curved and usually very hairy. Some types, however, have almost hairless pods. Seeds small, oblong, green or black with a concave hilum and no ridges on the seed coat.

There are two distinct varieties of *urid*, one with large black seeds, the other with somewhat smaller greenish seeds but no varietal names appear to have been given and hence it is proposed to include all the black-seeded *urid* in the Sub-variety *Niger*, Bose, and the green-seeded *urdi* in the Sub-variety *Viridis*, Bose. The green-seeded *urdi* has leaves of a lighter colour, and the pods lack the central dark stripe or vein which characterises the black variety. Prain [1898] points out that Linnaeus's description of *Phaseolus mungo* accords better with the *tikari* than with any related species. *Tikari kalai* is a scandent or subscandent herb and has a twining habit and is probably not grown outside India.

Habit.—The *urid* plants are generally very spreading and the branches usually are procumbent. In most types the trailing nature of the plants clearly distinguish this crop from the more or less semi-erect plants of *mung*. Only a very few types with rather scanty branching show a semi-erect habit. The branching may be scanty, or profuse.

Maturity.—The *urid* is a short-term crop taking only seven to eight weeks from sowing to flowering. Most of the black-seeded types usually ripen earlier than the green-seeded ones. The former ripen in August or September while the latter remain in the field till October or November. In the description of types three broad classes, *viz.*, early, medium early and late have been made by taking the average number of days taken by the first flower to open in the type as the criterion for gauging this character.

Stem.—The stem in *urid* is diffuse, furrowed and much branched from the base. It is densely clothed with long, brown hairs although the different types vary in the degree of hairiness. The colour is generally green with different amounts of diffused purple colour in the various unit species.

Leaves.—Leaves of this plant are trifoliate large ovate, and acuminate. The leaflets are membranous, wrinkled and hairy. The leaflets are broadly ovate or nearly rhomboid, orbicular, usually entire, thin, short and acute. Foliage colour is lighter than that of *mung* and is mostly light green. A few types with green and a very few types with dark green foliage, however, also exist. The size of the leaflets may be arbitrarily described as very large, large or small. The petiole is very long and hairy, chanelled and has diffused purple colour on it. At first petiole is longer than the peduncles but as the season advances the reverse is generally true.

Flowers.—Flowers are borne in capitate clusters on the ends of rather stout hairy peduncles. Usually there is only one flower stalk arising from the axil of each leaf but this stalk sometimes developes two or more branches. There is very little difference in the size of the flower in the different types of *urid* described in this paper. The standard is generally 12 to 16 mm. in width. Three grades of flower colour may be recognized but the differences are not very clear-cut and sometimes cause confusion. The flowers may be :—

1. Bright yellow. Example Type 19,
2. Lemon-yellow. Example Types 7, 20, etc., or
3. Pale yellow. Example Types 6, 13, etc.

In some types there are diffused red dots at the back of the standard, but as this is a minor character and has been found to be variable, it has not been used in the separation of different types.

Pods.—The pods of *urid* are much shorter, stouter and more hairy than those of *mung*. They are somewhat cylindrical and curved in shape. They are mostly

erect or sub-erect in contrast to the spreading or reflexed pods of *mung*. A few types have a nearly hairless or glabrous surface (Type 3) while the rest have hairy (Type 7) or very hairy (Type 17) surface. Definite variation in the colour of the ripe, as well as the unripe, pod can be recognized and the colour of the ripe pod has been utilized as one of the main characters for the separation of the different unit species. The following grades of colour have been observed :—

Colour of the ripe pods—	
1. Buff	Example Type 10
2. Brownish buff	„ „ 7
3. Brown	„ „ 14
4. Dark brown	„ „ 3
Colour of the unripe pods—	
1. Yellowish green	Example Type 10
2. Green	„ „ 7
3. Dark green	„ „ 3

Pods which have a yellowish green colour in the unripe condition generally have buff colour in the ripe stage. Green coloured pods ripen into brownish buff or brown colour, while dark green unripe pods show a brown or dark brown colour in the ripe stage, usually the latter.

Seed.—Urid has a small oblong seed about a sixth of an inch in length with almost flattened ends but at times these ends are rounded. The surface is usually dull and rough but may be slightly or fully lustrous in some types. The broad hilum is covered with a dense white caruncle grooved in the centre. The seed furnishes the most important character and is of the first magnitude in separating out the different types of *urid*. Two broad classes, *viz.*, green and black have been recognized from time immemorial and the *urid* crop is generally divided into these two varieties. A closer inspection, however, brings out differences in these two varieties themselves. The black colour of the *urid* is due to very closely packed dark flecks or marbling on a grey back ground, and the intensity of this marbling imparts to the grain different grades of black or dark colour. The following grades of seed colour have been used in the present classification.

	Types
I. *Green urdi* (*sub-variety* Viridis, *Bose*)—	
A. Seeds elm-green, dull	1 and 2
B. Seeds dark green—	
Shining	3 to 6
Dull	7 and 8
C. Seeds green—	
Shining	9
Dull	10 to 13
D. Seeds light green—	
Shining	14
Dull	15

II. *Black urid* (*sub-variety* Niger, *Bose*)—

E. Seeds with grey back-ground and very heavy black marbling, grade 1—

Shining	16
Shining somewhat	17 and 18
Dull	19 to 21

F. Seeds with grey back-ground and heavy black marbling, grade 2—

Shining somewhat	22 and 23
Dull	24

G. Seeds with dark green back-ground and sparse black marbling, grade 3—

Shining	25

IV. KEY TO THE TYPES OF *urid* (*Phaseolus mungo* LINN. VAR. *Roxburghii* PRAIN).

	Old culture No.	Type No.
I. *Green urdi* (*sub-variety* Viridis, *Bose*)		
A. Seeds elm-green		
Grains dull		
Flowers lemon-yellow		
Pods brown and hairy	S. M.	1
Flowers pale yellow		
Pods dark brown and hairless	L. M.	2
B. Seeds dark green		
Grains shining		
Flowers lemon-yellow		
Pods dark brown and hairless		
Medium in maturity		
Stem with light purple colour	G. 12	3
Stem with deep purple colour	L.	4
Late in maturity	G. 3	5
Flowers pale yellow		
Pods dark brown and hairless	G. 1	6
Grains dull		
Flowers lemon-yellow		
Pods brownish buff and hairy	G. 4	7
Flowers pale yellow		
Pods brownish buff and hairy	G. 22	8

PLATE LXX.

FLOWER COLOUR.

Fig. 1.—Standard colour—*Pale yellow.* Type 6.

Fig. 2.—Standard colour—*Lemon-yellow.* Type 10.

POD COLOUR.

Fig. 3.—Unripe pod—*Yellowish green.* Type 10.

Fig. 4.—Unripe pod—*Green.* Type 7.

Fig. 5.—Unripe pod—*Dark green.* Type 3.

Fig. 6.—Ripe pod—*Dark brown.* Type 3.

Fig. 7.—Ripe pod—*Buff.* Type 10.

SEED COLOUR.

Fig. 8.—Seeds—*Light green, dull.* Type 15.

Fig. 9.—Seeds—*Dark green, dull.* Type 8.

Fig. 10.—Seeds—*Light green, shining.* Type 14.

Fig. 11.—Seeds—*Dark green, shining.* Type 4.

Fig. 12.—Seeds—*Elm-green,* Type 1.

Fig. 13.—Seeds—*Dark green with sparse black marbling, shining.* Type 25.

Fig. 14.—Seeds—*Grey background with heavy black marbling, dull.* Type 20.

Fig. 15.—Seeds—*Grey background with very heavy black marbling, dull.* Type 21.

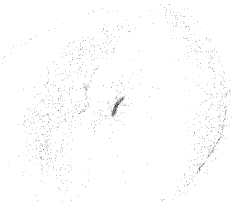
Fig. 16.—Seeds—*Grey background with very heavy black marbling, shining.* Type 16.



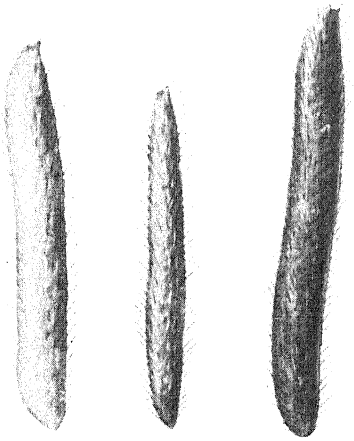




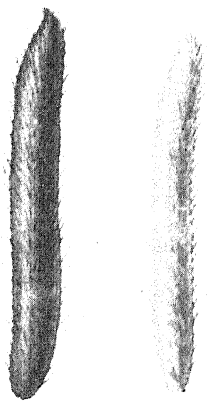
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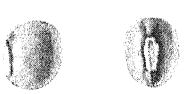
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3. 4. 5.



6. 7.



8.



9.



10.



11.



12.



13.



14.

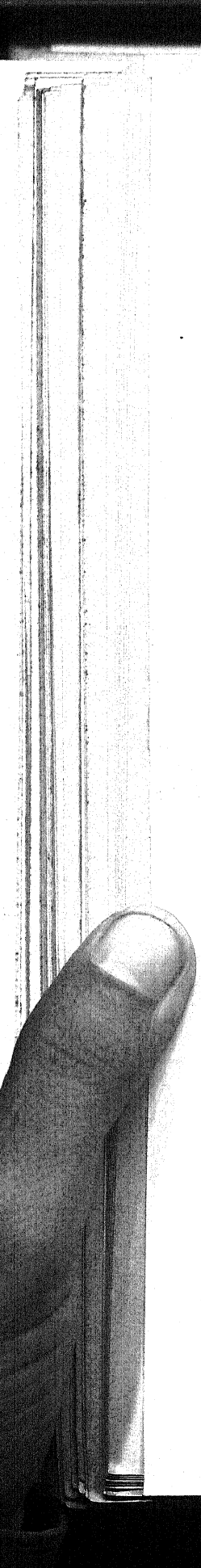


15.



16.

Flower, pod and seed colour in *urid*.



IV. KEY TO THE TYPES OF *urid* (*Phaseolus mungo* LINN. VAR. *Roxburghii* PRAIN)—*contd.*

	Old culture No.	Type No.
I. *Green urdi* (*sub-variety Viridis, Bose*)—contd.		
C. Seeds green		
Grains shining		
Flowers lemon-yellow		
Pods dark brown and hairless . .	G. 2	9
Grains dull		
Flowers lemon-yellow		
Pods buff and hairy	G. 26	10
Pods brownish buff and hairy . .	G. 23-2	11
Pods dark brown and hairless . .	H. 2	12
Flowers pale yellow		
Pods brownish buff and hairy . .	G. 29	13
D. Seeds light green		
Grains shining		
Flowers lemon-yellow		
Pods brown and hairless . . .	G. 20	14
Grains dull		
Flowers lemon-yellow		
Pods buff and hairy	G. 13	15
II. *Black urid* (*sub-variety* Niger, *Bose*)		
E. Seeds with grey back-ground and very heavy black marbling, grade I.		
Grains shining		
Flowers lemon-yellow		
Pods brownish buff and hairy . .	K. 3	16
Grains shining somewhat		
Flowers lemon-yellow		
Pods brown and very hairy . . .	B. 50	17
Pods dark brown and hairless . . .	B. 80	18

IV. KEY TO THE TYPES OF *urid* (*Phaseolus mungo* LINN. VAR. *Roxburghii* PRAIN)—*contd.*

	Old culture No.	Type No.
II. *Black urid* (*sub-variety* Niger, *Bose*)—contd.		
E. Seeds with grey back-ground and very heavy black marbling, grade 1—*contd.*		
Grains dull		
Flowers bright yellow		
Pods dark brown and hairy . . .	B. 7	19
Flowers lemon-yellow		
Pods brown and hairy		
Habit erect	B. 76	20
Habit spreading	B. 28	21
F. Seeds with grey back-ground and heavy black marbling, grade 2. Grains shining somewhat		
Flowers lemon-yellow		
Pods brown and hairy		
Early in maturity	B. 30	22
Medium in maturity	B. 69	23
Grains dull		
Flowers lemon-yellow		
Pods brown and hairy	B. 63 E.	24
G. Seeds with dark green back-ground and sparse black marbling, grade 3. Grain shining		
Flowers pale yellow		
Pods brownish buff and hairless . .	K. 1	25

V. DESCRIPTION OF *urid* TYPES.

1. Urdi *or green-seeded types, sub-variety*—Viridis (*Bose*).

Type 1.—Late, spreading, profuse branching. *Stem* green with purplish splashes, very hairy. *Leaves* large; foliage green. Flowers lemon-yellow. *Pods* hairy; unripe—yellowish green; ripe—brown. *Seeds* elm-green, dull.

Type 2.—Late, trailing, profuse branching. *Stem* green with purplish splashes, hairy. *Leaves* large; foliage green. *Flowers* pale yellow. *Pods* hairless; unripe—yellowish-green; ripe—dark brown. *Seeds* elm-green, dull.

Type 3.—Medium in maturity, profuse branching, branches thin. *Stem* green with a few purplish splashes, the purple colour almost disappearing in the mature stage, very slightly hairy. *Leaves* large; foliage green. *Flowers* lemon-yellow. *Pods* hairless; unripe—dark green; ripe—dark brown. *Seeds* dark green, shining.

Type 4.—Medium in maturity, trailing, profuse branching. *Stem* green with purplish splashes, hairless. *Leaves* large; foliage green. *Flowers* lemon-yellow. *Pods* hairless; unripe—dark green; ripe—dark brown. *Seeds* dark green, shining.

Type 5.—Late, spreading, very profuse branching. *Stem* green with purplish splashes, slightly hairy. *Leaves* large; foliage green. *Flowers* lemon-yellow. *Pods* hairy; unripe—green; ripe—brownish buff. *Seeds* dark green, shining.

Type 6.—Medium in maturity, spreading, profuse branching, thin branches. *Stem* green with a few purplish splashes, slightly hairy. *Leaves* large; foliage green. *Flowers* pale yellow. *Pods* hairless; unripe—dark green; ripe—dark brown. *Seeds* dark green, shining.

Type 7.—Medium in maturity, spreading, profuse branching. *Stem* green with purplish splashes, slightly hairy. *Leaves* large; foliage light green. *Flowers* lemon-yellow. *Pods* hairy; unripe—green; ripe—brownish buff. *Seeds* dark green, dull.

Type 8.—Early, spreading, profuse branching. *Stem* green with a few purplish splashes, hairy. *Leaves* large; foliage light green. *Flowers* pale yellow. *Pods* hairy; unripe—green; ripe—brownish buff. *Seeds* dark green, dull.

Type 9.—Medium in maturity, spreading, profuse branching. *Stem* green with purplish splashes, slightly hairy. *Leaves* large, foliage green. *Flowers* lemon-yellow. *Pods* hairless; unripe—dark green; ripe—dark brown. *Seeds* green, shining.

Type 10.—Medium in maturity, spreading, very profuse branching. *Stem* green with purplish splashes, hairy. *Leaves* large; foliage light green. *Flowers* lemon-yellow. *Pods* hairy; unripe—yellowish green; ripe—buff. *Seeds* green, dull.

Type 11.—Medium in maturity, spreading, profuse branching. *Stem* green with purplish splashes, hairy. *Leaves* rather large; foliage green. *Flowers* lemon-yellow. *Pods* hairy; unripe—green; ripe—brownish buff. *Seeds* green, dull.

Type 12.—Late, spreading, profuse branching. *Stem* green with purplish splashes, slightly hairy. *Leaves* large; foliage dark green. *Flowers* lemon-yellow. *Pods* hairless; unripe—dark green; ripe—dark brown. *Seeds* green, dull.

Type 13.—Early, spreading, moderate branching. *Stem* green with purplish splashes, hairy. *Leaves* large; foliage light green. *Flowers* pale yellow. *Pods* hairy; unripe—green; ripe—brownish buff. *Seeds* green, dull.

Type 14.—Late, trailing, profuse branching. *Stem* green with purplish splashes, slightly hairy. *Leaves* large, foliage green. *Flowers* lemon-yellow. *Pods* hairless; unripe—dark green; ripe—brown. *Seeds* light green, shining.

Type 15.—Late, spreading, profuse branching. *Stem* green with purplish splashes, hairy. *Leaves* large; foliage light green. *Flowers* lemon-yellow. *Pods* hairy; unripe—yellowish green; ripe—buff. *Seeds* light green, dull.

2. Urid *or black-seeded types, sub-variety*—Niger (*Bose*).

Type 16.—Medium in maturity, spreading, moderate branching. *Stem* green with purplish splashes, hairy. *Leaves* rather large; foliage green. *Flowers* lemon-yellow. *Pods* hairy; unripe—yellowish green; ripe—brownish buff. *Seeds* grey back-ground with very heavy black marbling, grade 1, shining.

Type 17.—Medium in maturity, spreading, very profuse branching. *Stem* green with purplish splashes, very hairy. *Leaves* large; foliage light green. *Flowers* lemon-yellow. *Pods* very hairy; unripe—green; ripe—brown. *Seeds* grey back-ground with very heavy black marbling, grade 1, shining somewhat.

Type 18.—Medium in maturity, spreading, profuse branching. *Stem* green with purplish splashes, slightly hairy. *Leaves* rather large; foliage light green. *Flowers* lemon-yellow. *Pods* hairless; unripe—dark green; ripe—dark brown. *Seeds* grey back-ground with very heavy black marbling, grade 1, shining somewhat. The hilum has a greenish border.

Type 19.—Medium in maturity, spreading, moderate branching. *Stem* green with purplish splashes, hairy. *Leaves* small; foliage light green. *Flowers* bright yellow. *Pods* hairy; unripe—dark green; ripe—dark brown. *Seeds* grey back-ground with very heavy black marbling, grade 1, dull.

Type 20.—Medium in maturity, semi-erect habit, moderate branching. *Stem* green with purplish splashes, hairy. *Leaves* rather large; foliage dark green. *Flowers* lemon-yellow. *Pods* hairy; unripe—green; ripe—brown. *Seeds* grey back-ground with very heavy black marbling, grade 1, dull.

Type 21.—Medium in maturity, spreading, profuse branching. *Stem* green with purplish splashes, hairy. *Leaves* rather large, foliage light green. *Flowers* lemon-yellow. *Pods* hairy; unripe—green; ripe—brown. *Seeds* grey back-ground with very heavy black marbling, grade 1, dull.

Type 22.—Early, spreading, scanty branching. *Stem* green with purplish splashes, hairy. *Leaves* small; foliage light green. *Flowers* lemon-yellow. *Pods*

hairy; unripe—green; ripe—brown. *Seeds* grey back-ground with heavy black marbling, grade 2, shining somewhat.

Type 23.—Medium in maturity, trailing, very profuse branching. *Stem* green with purplish splashes, very hairy. *Leaves* large; foliage light green. *Flowers* lemon-yellow. *Pods* hairy; unripe—green; ripe—brown. *Seeds* grey back-ground with heavy black marbling, grade 2, shining somewhat.

Type 24.—Medium in maturity, semi-erect, profuse branching. *Stem* green with purplish splashes, hairy. *Leaves* rather large, foliage dark green. *Flowers* lemon-yellow. *Pods* hairy; unripe—green; ripe—brown. *Seeds* grey back-ground with heavy black marbling, grade 2, dull.

Type 25.—Late, spreading, moderate branching. *Stem* green with purplish splashes, slightly hairy. *Leaves* large, foliage green. *Flowers* pale yellow. *Pods* hairless; unripe—yellowish green; ripe—brownish buff. *Seeds* dark green back-ground with sparse black marbling, grade 3, shining.

VI. REFERENCES.

Bose, R. D. (1932). *Ind. J. Agric. Sci.* **2**, 607-624.
Butler, E. J. (1918). Fungi and Disease in Plants.
Church, A. H. (1886). Food Grains of India, 151.
Ghosh, M. N., and Basu, S. K. (1925). *Agric. J. Ind.* **20**, 147-148.
Leather, J. W. (1903). *Agric. Ledger* No. **7**, 152-155.
Murray, F. (1892). Dic. Econ. Prod. **6**, 193.
Narasimham, M. (1929). *Agric. J. Ind.* **24**, 397-401.
Prain, D. (1898). *J. Asiatic Soc. of Bengal*, **66**, 422.
Piper, C. V. (1915). Forage Plants and their Culture, New York, 552.
Watt, G. (1908). Common Products of India, 880-881.

ON THE NATURE OF THE REACTIONS RESPONSIBLE FOR SOIL ACIDITY.*

PART II.—TITRATION CURVES OF SILICIC ACID SOL, HUMIC ACID SOL AND ALUMINIUM HYDROXIDE SOL.

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(With 15 text-figs.)

In a previous paper [Mukherjee and Sen, 1931] the titration curves of sparingly soluble acids (cinnamic, isophthalic and p-toluic acids) with or without excess of solid phase have been given. These curves show one dissociation constant inspite of the particles having dimensions much larger than what is usual with colloidal particles. In connection with the calculation of the dissociation constant and the interpretation of the curves which underlie such calculations, the shortcomings of the usual interpretations of titration curves of soils, of colloidal acid clay and similar systems have been briefly indicated. The dissociation constants were calculated according to the simplified formula $pH = pK + \log \frac{[Salt]}{[Acid]}$.

In the case of a colloidal solution of aluminium hydroxide it was shown that traces of electrolytes present exert a great influence on the behaviour of the sol. Silica and alumina are the chief constituents of alumino-silicates and further work has been done with sols of these two substances. Potentiometric and conductometric titrations of a humic acid sol have also been carried out.

It has been indicated in the introduction to Part I that systems of colloidal acids or bases require a theoretical treatment different from that of acids and bases in 'true' solution. It will be seen from the titration curves of silicic and humic acids that such a differentiation is called for. One of the main results so far obtained is that these colloidal solutions of acid substances have in many respects the properties of a strong acid and in fact the properties of an ultra strong acid. The latter statement is made with some reserve pending further work. The importance of traces of electrolytes specially of carbon dioxide from the air is often overlooked in the work on soil systems, and attention has been drawn to the magnitude of the effect it may exert on the observations. The experimental work in these series of papers is closely related to some fundamental problems in

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colloid chemical analysis [Mukherjee, Roychoudhury and Biswas, 1931; Weiser, 1931; Rabinowitsch and Kargan, 1928; Wassilew and Rabinowitsch, 1931; Wintgen and Biltz, 1923; Wintgen and Kuhn, 1928; Muttone and Pauli, 1931], and for building up a rational basis for the interpretations of the data attention might with advantage be paid to the need for simultaneous measurements of several types. An arrangement* which admits of such simultaneous measurements has been described and results obtained with an aluminium hydroxide sol have been given.

Experimental.

Preparation of the sols.

(1) *Silicic acid sol.*—Thirty cubic centimeter of hydrochloric acid (*Kahlbaum, Pro Analysi*) were diluted with 100 c.c. of water. 75 c.c. of sodium silicate solution (density 1·16) were poured into it accompanied by stirring, the mixture was dialysed in a parchment bag against repeated changes of distilled water till the sol had become slightly turbid (between pH 3·0 to pH 4·0). The sol was stocked in a Jena glass bottle. This sol remained stable without setting for about seven days. Three different sols (I, II, III) were prepared in this manner for this work. The sols had different silica contents. Coagulated masses of silica were noticeable after sometime.

(2) *Humic acid sol.*—Five grams of Merck's humic acid were taken in a 2 litre Jena glass bottle and shaken with 2 litres of conductivity water for 6 hours and allowed to rest for 3 days during which the coarser particles settled below leaving a stable sol. No sediment deposited during the period of work.

(3) *Aluminium hydroxide sol.*—A litre of 0·1 *N* ammonia solution was added with constant stirring to a litre of 0·1 *N* aluminium chloride solution. The precipitate was washed at first by decantation and then by the help of a centrifuge. The precipitate began to be peptised gradually. After a few washings by the centrifuge the final precipitate which remained gave a fairly finely dispersed sol when suspended in water. This sol had a slight tendency to settle. After a few days the upper finely-dispersed portion was siphoned off into another bottle. The sol thus obtained was very stable and showed no sign of settling for several months.

Stocking of the aluminium hydroxide sol.

This sol had a conductivity comparable to that of "conductivity" water. The following procedure was adopted to free it from dissolved carbon dioxide and to keep it free from carbon dioxide and oxygen of the air :—The sol was heated on a water bath for 4 hours and cooled in a Jena glass bottle fitted with a cork with three holes, through one of which a long vertical tube was inserted.

* This arrangement and the results with it have been arrived at in collaboration with Saroj Kumar Dasgupta and Ashutosh Chatterjee who were working on colloid chemical analysis in this laboratory. This portion has been also separately communicated for publication in connection with their work.

This tube was bent twice at its top at right angles and the other end passed through a cork fitted to the top of a burette which was connected to a suction pump (Fig. 1). Through the other two holes two bent glass tubes fitted with glass stopcocks permitted respectively the inlet and outlet of pure hydrogen. The cooling took place while a constant stream of pure hydrogen passed through the sol. The hydrogen stream was stopped when the sol attained the temperature of the room (about 5 hours). Samples were taken from this stock sol by passing hydrogen through the inlet tube and suction from a small pump applied at the top of the burette. The sol collects in the burette. The inlet tube of hydrogen is closed and hydrogen passed through the top of the burette so as to allow the sol to drain through the burette as usual.

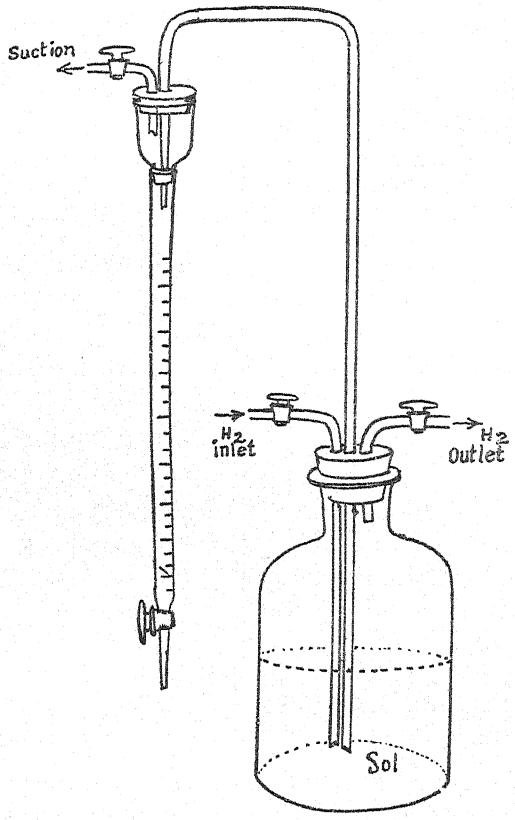


Fig. 1.

Potentiometric titrations.

The potentiometric titrations were carried out in an arrangement shown in Figure 2 with a baryta solution of known strength in a microburette. The titration vessels which contained a definite volume of the sol consisted of a wide mouthed Jena glass bottle provided with a tightly fitting rubber cork through which five holes were bored for admitting (1) the stem of the burette, (2) the platinum electrode, (3) the hydrogen inlet, (4) a stirrer and (5) a bent glass tube provided with a stopcock. The last secured connection with a second wide-mouthed Jena glass bottle containing the same sol, and was connected in turn by means of a similar bent tube with a saturated KCl solution in a beaker. Hydrogen was also passed into the second vessel containing the sol. The e. m. f. was measured against a normal calomel electrode. All measurements were carried out in a thermostat whose temperature remained constant at 35±·1°C.

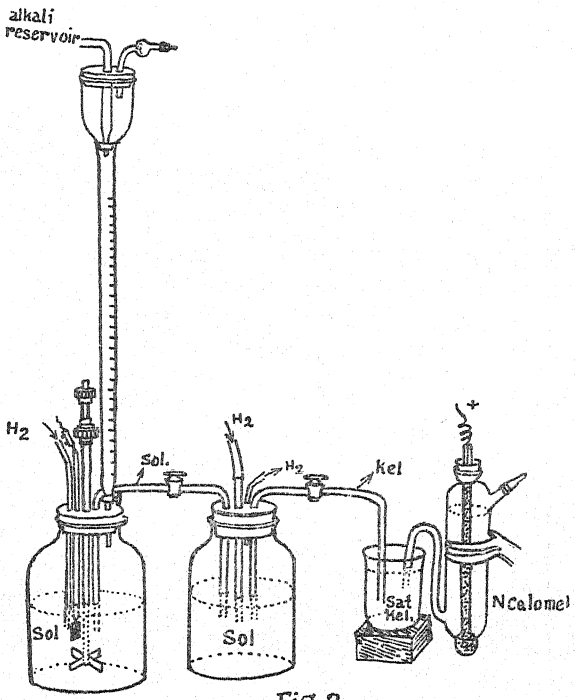


Fig. 2.
Potentiometric Titration

Conductometric titrations.

The conductometric titrations were carried out with a Leeds Northrup 'student type' conductivity bridge calibrated *i.e.*, the errors for different readings are known. The sol was taken in a microburette and added in instalments to a baryta solution contained in a wide mouthed 100 c.c. Jena glass bottle fitted with a rubber cork with four holes for admitting (1) the burette, (2) the two electrodes, (3) a stirrer, and (4) a tube for passing hydrogen (Fig. 3). The thermostat was maintained at 35± 1°C.

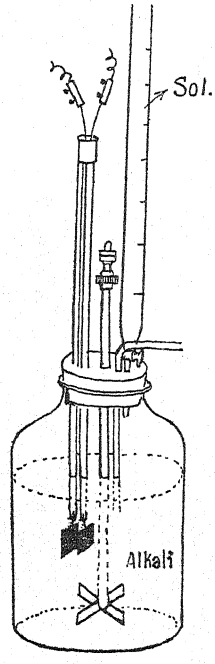


Fig. 3.
Conductometric Titration

Estimation of carbonate.

The amount of carbon dioxide present in the sol, in spite of these precautions, was estimated as follows (Fig. 4) :—

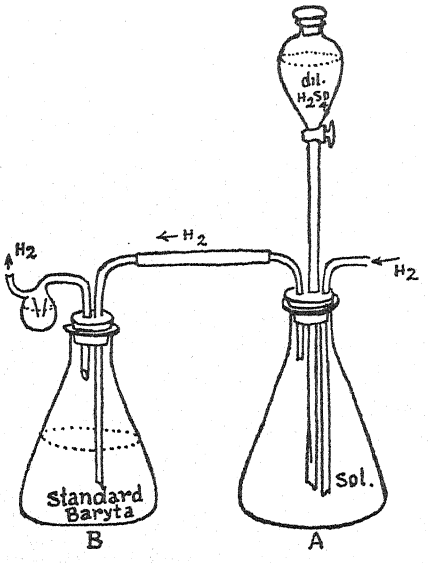


Fig. 4.

A 150 c.c. conical flask (A) was provided with an air-tight rubber cork provided with three holes, respectively for the inlet and outlet of pure hydrogen and for the introduction of a small separating funnel. The flask containing a definite amount of the liquid was gently heated on a water bath while a slow stream of pure hydrogen passed through it and chased out the carbon dioxide. The outlet was connected to a conical flask (B) provided with an air-tight rubber cork and containing a known volume of baryta solution of ascertained strength. The current of hydrogen was continued through 'A' long enough to chase out all the carbon dioxide. The bulb and the stem of the separating funnel fitted to 'A' contained dilute sulphuric acid. After the carbon dioxide has been chased out for sometime, dilute sulphuric acid was poured into 'A' from the separating funnel, thus liberating carbon dioxide from any carbonate that may be present. The baryta was titrated against a standard solution of HCl using methyl red as indicator with usual precautions.

An improved titration vessel, suitable for our purpose, which admits of simultaneous measurements was used for both potentiometric and conductometric titrations in the latter part of our work (Fig. 5). An Erlenmeyer flask of pyrex

glass was sealed a little above its open end and *seven* air-tight ground-in joints admitted *a* ground-in thermometer stem and *five* ground-in pyrex glass tubes, with appropriate fittings, which serve as follows :—(*a*) An inlet for hydrogen gas ; (*b*) an internally sealed double tube containing platinum electrodes suitable for conductivity measurements ; the electrodes were fixed in position with sealed glass rods so that they remain parallel and at a fixed distance apart ; (*c*) a platinum electrode sealed at the end of a glass tube used as hydrogen electrode ; (*d*) secures a connection with a large vessel containing saturated potassium nitrate* ; (*e*) an Ag/AgCl electrode. Through the *seventh* opening passed a tight fitting rubber cork containing two holes,

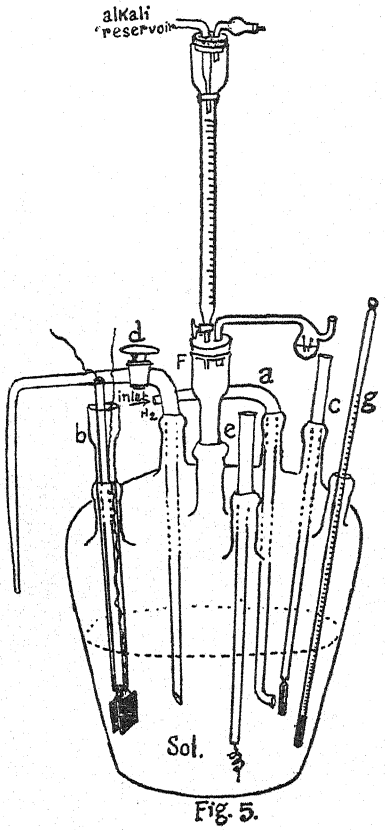


Fig. 5.

* The tips of a normal calomel electrode for measurement of the hydrogen ion concentration and of a vessel containing an Ago/AgCl electrode in 0·01 *N* HCl were dipped into the potassium nitrate solution.

through one of which was inserted the mouth of a burette, while through the other passed the tip of a gas trap which served as the outlet of hydrogen. This vessel allows the change in hydrogen ion concentration, chlorine ion concentration and conductivity to be followed with the gradual addition of baryta free from carbon dioxide in an atmosphere of pure hydrogen as also other types of simultaneous measurements, sometimes necessary in analysis of colloidal systems, where ageing and other changes in the sol render it most desirable to take all relevant measurements at the same time.

Estimation of total chlorine.

The total chlorine contents of the sols were determined by means of silver-silver chloride electrodes (prepared as given by Noyes and Ellis, 1917), by electrometric titration using a saturated solution of silver chloride for the reference electrode. A measured volume of the sol was dissolved in nitric acid. To this solution solid potassium nitrate was added so as to make its concentration 5 per cent. and titrated with a centinormal $AgNO_3$ solution from a microburette. The titration was continued after the change in the sign of the e. m. f. The total chlorine was calculated after plotting the results in a curve.

Chlorine ion concentration.

The chlorine ion concentration was determined by means of silver-silver chloride electrodes previously prepared. Two of these electrodes were dipped respectively into a KCl solution of known strength and into the colloidal solution under investigation. Connection between the two was secured through a saturated solution of potassium nitrate.

Reliability of the Ag-AgCl electrodes used.

The reliability of the silver-silver chloride electrodes was tested carefully. When not in use the electrodes were kept in a vessel wrapped with black paper. The following Table will show the reliability of the Ag-AgCl electrodes.

$1{\cdot}75\frac{N}{50}$ HCl | Ag-AgCl | — ——— Sat. KNO_3 ——— $1{\cdot}75\frac{N}{250}$ HCl | Ag-AgCl | +

Observed e.m.f. of the above cell = 0·0405 volt.

Also e.m. f. calculated from $\frac{RT}{F}$ log $\frac{\alpha_1 C_1}{\alpha_2 C_2}$ = 0·0397 volt (from the figures given by Randall and Young, 1928).

Preparation of hydrogen (Fig. 6).

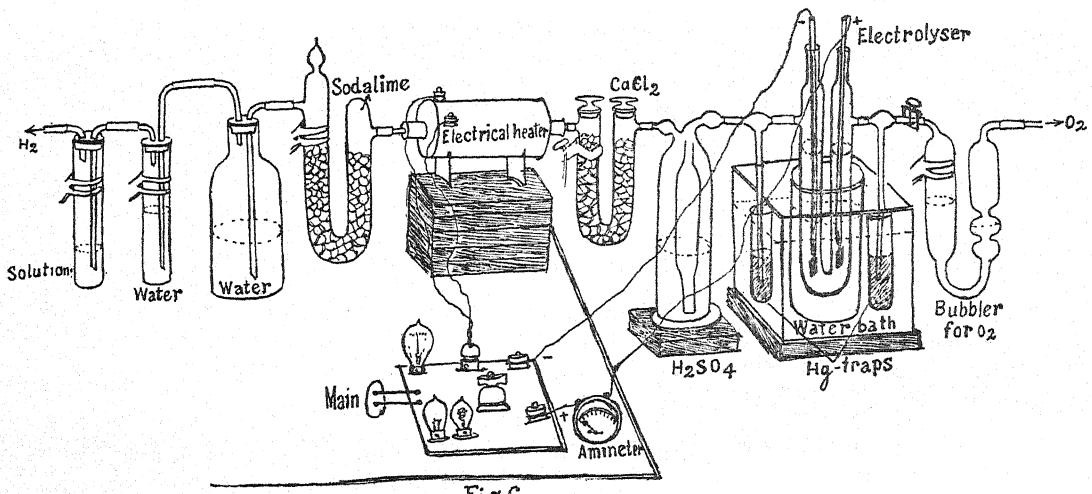


Fig 6.

The hydrogen was obtained from the electrolysis of caustic soda solution with platinum electrodes and freed from oxygen by passing the gas dried over sulphuric acid and calcium chloride through copper gauge heated electrically to about 400°C. The gas was then passed through soda lime and then through bubblers containing respectively pure water and the solution to be used. The conductivity of the pure water was tested at intervals to ascertain the absence of electrolytic impurities in the gases. In the first portion of the work electrically heated platinum wires were used instead of the copper gauze.

A.—Silicic acid sol.

(*i*) *Potentiometric titration of silicic acid sol.*—Successive readings were taken at half an hour intervals which was necessary for the attainment of an equilibrium value of the e.m.f. 75 c.c. of the sol was taken in each case.

TABLE I.

(*Strength of baryta solution =0·1815 N.*)

Sol I (Curve I)		Sol II (Curve II)		Sol II (Curve III) (Repetition of Curve II)	
Alkali	pH	Alkali	pH	Alkali	pH
c.c.		c.c.		c.c.	
0	2·63	0	4·17	0	4·13
0·2	2·70	0·2	5·30	0·15	4·64
0·4	2·78	0·3	5·77	0·2	4·89
0·6	2·90	0·4	6·13	0·25	5·17
0·8	3·02	0·5	6·64	0·3	5·36
1·0	3·13	0·7	6·92	0·5	6·06
1·2	3·31	0·8	7·00	0·6	6·38
1·3	3·50	0·9	7·03	0·7	6·70
1·4	3·77	1·0	7·17	0·8	6·93
1·45	5·06	1·1	7·23	0·9	7·01
1·5	5·22	1·6	7·35	1·0	7·09
1·55	5·33	1·7	7·41	1·1	7·17
1·65	5·44	..	..	1·2	7·25
1·8	5·51	..	..	1·3	7·33
2·1	5·74	..	..	1·4	7·42
2·5	6·71	..	..	1·5	7·50
2·7	7·12	..	..	1·6	7·55
2·9	7·28	..	..	1·8	7·65
3·1	7·40	..	..	2·0	7·75
3·3	7·51	..	..	..	..

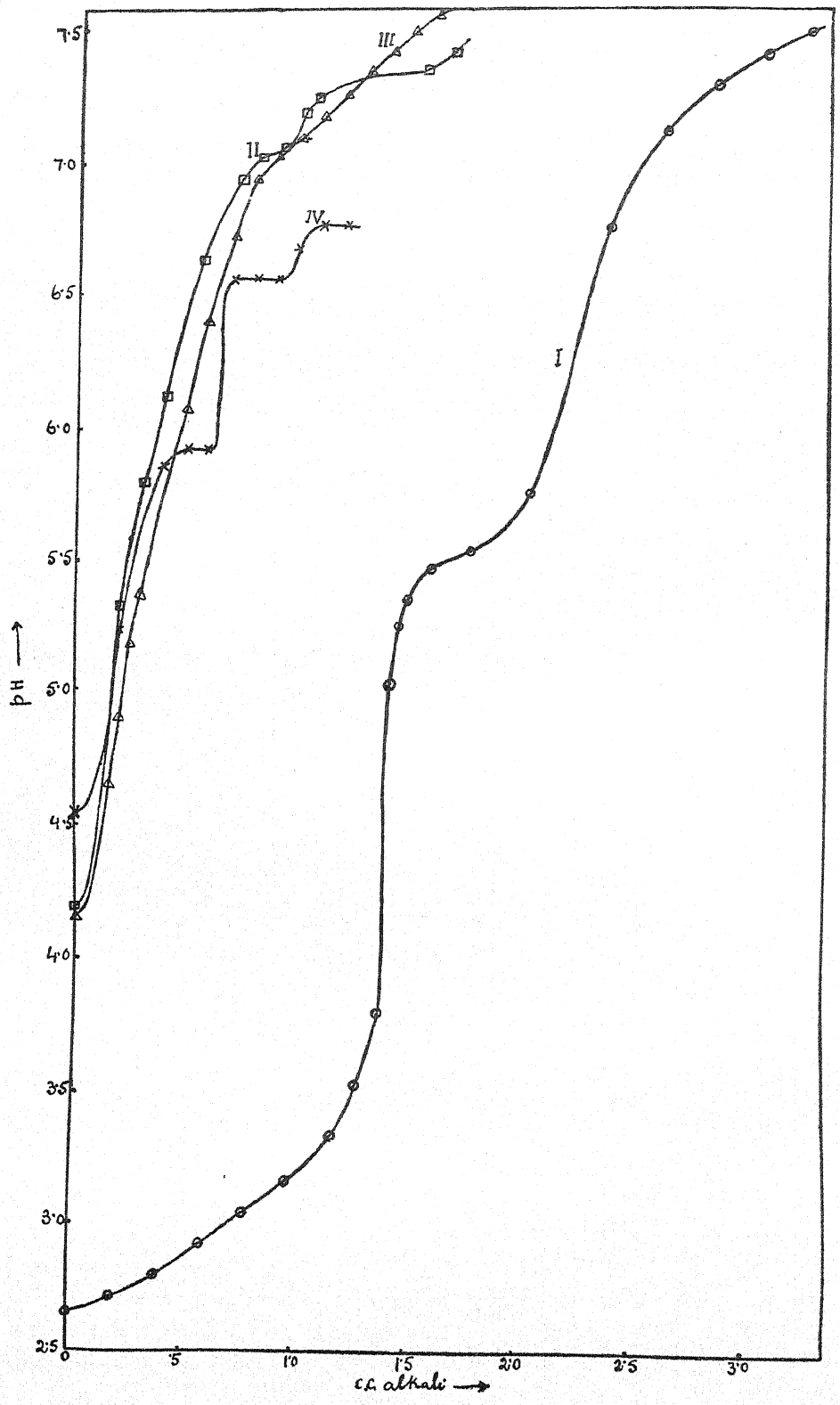

7·5
7·0
6·5
6·0
5·5
5·0
4·5
4·0
3·5
3·0
2·5
pH
0
·5
1·0
1·5
2·0
2·5
3·0
c.c. alkali
I
II
III
IV

Fig. 7.

TABLE II.

(Strength of baryta solution =0·00927 N.)

Sol II (1:1, *i.e.*, diluted with an equal vol. of water Curve IV)		Sol III (1:1, Curve V)		Sol III (1:4, Curve VI)		Sol III (1:4, Curve VII) (Repetition of Curve VI)	
Alkali	pH	Alkali	pH	Alkali	pH	Alkali	PH
c.c.		c.c.		c.c.		c.c.	
0	4·54	0	3·00	0	3·67	0	3·70
0·2	5·23	1·0	3·49	0·4	3·92	0·5	3·97
0·4	5·84	2·5	3·81	1·0	4·21	1·0	4·25
0·5	5·91	3·0	3·81	1·6	5·07	1·5	4·77
0·6	5·91	3·5	3·87	1·8	5·23	2·0	5·77
0·7	6·55	4·0	4·04	2·0	5·63	2·5	7·07
0·8	6·55	4·5	4·20	2·2	6·02	3·0	7·47
0·9	6·55	5·0	4·73	2·4	6·22	..	..
1·0	6·67	5·5	5·05	2·8	6·51	..	..
1·1	6·75	6·5	5·63	3·0	6·62	..	..
1·2	6·75	7·0	5·72	..	..	..	..
..	..	7·5	5·74	..	..	..	..
..	..	8·0	5·79	..	..	..	..
..	..	8·5	6·00	..	..	..	..
..	..	9·5	6·48	..	..	..	..
..	..	10·0	6·53	..	..	..	..
..	..	10·5	6·67	..	..	..	..
..	..	11·5	6·79	..	..	..	..
..	..	12·5	6·80	..	..	..	..
..	..	13·0	6·80	..	..	..	..

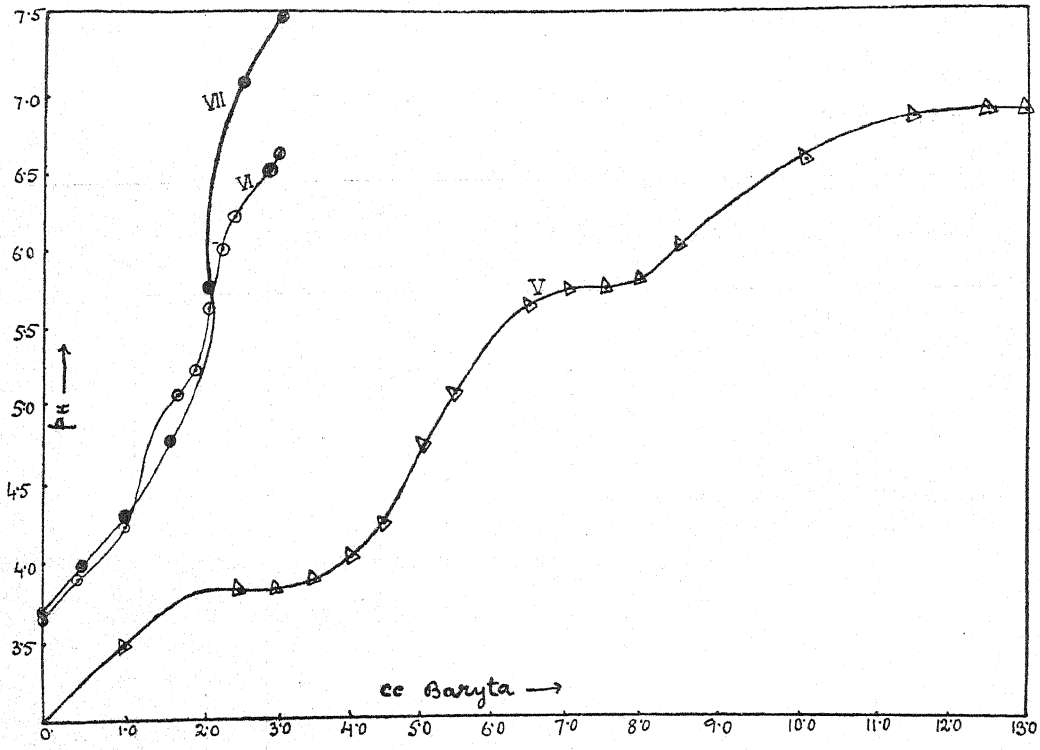


Fig. 8.

(*ii*) *Conductometric titration of silicic acid sol.*—Successive readings were taken at half an hour intervals to make sure that equilibrium was attained. 50 c.c.s. of 0·000927 *N* baryta solution were taken in the conductivity vessel and silicic acid sol (III) was added from the burette.

TABLE III.

(Curve VIII)	
Sol	Sp. conductivity × 10^4
c.c.	
0	2·083
0·25	1·909
0·5	1·574
0·75	1·374
1·0	1·179
1·25	1·116
1·5	1·097
2·0	1·075
2·5	1·150
3·5	1·373
4·0	1·513
5·5	2·345
6·5	2·665
7·5	3·064
8·5	3·414

Potentiometric titration

Curve I was obtained by titration with 0·1815 *N* baryta. It has the form of a titration curve of a dibasic acid. On closer examination it is found that the time of contact is a great factor in determining the slope of these titration curves. With a slow rate of addition of the alkali the rate of rise of pH diminishes (*vide* Curves II, III, VI and VII). Comparing Curves VI and VII we find that on the addition of the same amount of alkali (3 c.c.) to a definite volume of a particular silicic acid sol the pH increases from 3·67 to 6·62 in Curve VI when the alkali has been added in nine instalments (taking 4½ hours) while in Curve VII the pH increase is from 3·70 to 7·47 where the alkali has been added in six instalments (taking 3 hours). Curves VI and VII show that even the form of the curve materially depends on the manner of the addition of the alkali. Curve VII shows for the same amount of alkali added only one stage of neutralisation and that a fairly strong acid is being neutralised. This is an important point to be remembered and will be referred to later.

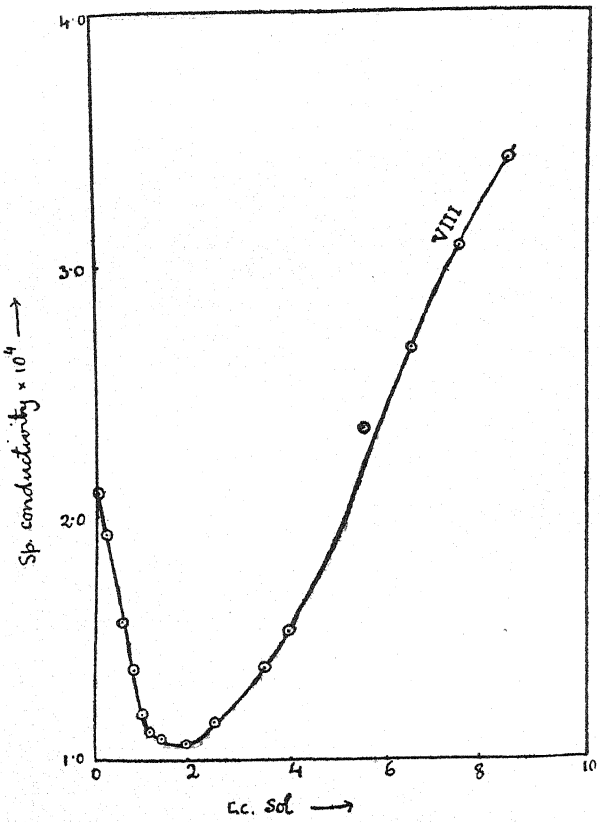


Fig. 9.

Attention has already been drawn [Mukherjee and Sen, 1931] to the role of small amounts of electrolytes in these titrations. The quantities of acids and bases involved in these sols are small. Apart from the effect of the possible variation in the amount of electrolytes taking part in the reaction, the colloidal system itself changes with time. The question of the reproducibility of the results therefore deserves special attention. With usual precautions the results are not

always exactly reproducible (Curves II and III, VI and VII). With silicic acid sols visible flakes appear with time. In the case of a particular sol of silicic acid it set on the addition of a certain minimum amount of alkali indicating that the acidity of the sol near the electrode remains unaffected. The measurements with this sol were discontinued. This observation requires further attention. These considerations show the need for circumspection regarding the technique and interpretation of the data in such measurements (see Curve IV in particular).

A rough estimate of the dissociation constants, assuming that monobasic weak acids are dealt with, can be formed from the total acidity indicated by the curve. The total acidities and the dissociation constants corresponding to the two stages of neutralisation for the different curves are given below.

TABLE IV.

Curve No.	Total acidity		Dissociation constants	
	1st stage	2nd stage	1st stage	2nd stage
I	0·0034 N	0·0054 N	$6{\cdot}33\times10^{-4}$	$1{\cdot}35\times10^{-5}$
V	0·00068 N	0·0011 N	$1{\cdot}63\times10^{-4}$	$\cdot59\times10^{-5}$
VI	0·00017 N	0·00024 N	$1{\cdot}56\times10^{-4}$	$3{\cdot}85\times10^{-5}$

The role that may be played by the carbon dioxide from the atmosphere and that present in the sol should not be overlooked and hence measurements should be carried out after eliminating as far as possible this source of disturbance. The significance of these dissociation constants and the theoretical aspects of such calculations will be dealt with in a later paper. The initial and final pH values in the titration curves have not been used and the figures given indicate the average of those values which lie within that stretch of the curve which gives fair agreement between the calculated constants. The conductometric and potentiometric curves give different amounts of total acidity as shown in Table V.

TABLE V.

Sol	Titration	Curve number	Total acidity
Silicic I	Potentiometric	I	0·003448 N*
			0·00544 N
,, II (1:1)	,,	IV	0·00008034 N
			0·0006798 N*
,, III (1:1)	,,	V	0·001125 N
			0·0001668 N*
,, III (1:4)	,,	VI	0·0002472 N
,, III (1:4)	,,	VII	0·0002619 N
,, III	Conductometric	VIII	0·02806 N
**Humic I	Potentiometric	XII	0·00007168 N
**Humic I (1:1)	Conductometric	XIII	0·002208 N

* The two values in these cases represent total acidities as calculated from the two stages of neutralisation respectively.

** Cf. Tables VII and VIII (Curves XII and XIII) given later.

The conductometric titration also shows that we are dealing with a fairly strong acid. This is in agreement with the observations of Rabinowitsch and Laskin [1928] on siticic acid sols. The conductometric titrations give total acidities which are several times greater than that obtained from potentiometric titrations. The values obtained from the latter are in turn much greater compared to the free acidities indicated by the pH values. This implies on the other hand on the basis of analogy with solutions of true acids a low degree of dissociation. The steepness of the rise after neutralisation of the sp. conductivity is striking in this connection (*vide* also Rabinowitsch and Laskin, (loc.-cit.). The presence of neutral salts of the colloidal acidic substance formed by the interaction does not seem to have so pronounced an effect on the increase in hydrogen ion concentration on further additions of the acid as one would expect from the interaction under similar conditions between a weak acid and its salt. In fact the rise shows that hydrogen ion concentration is almost proportional to the amount of 'acid' added which can only be the case for a very strong acid. The great difference in the total acidities obtained by the two methods can be explained as being due to the fact that in the case of the conductometric titration we are adding small volumes of the sol from the burette so that in this case the pH has a value always on the alkaline side; consequently all the hydrogen ions associated with the colloidal particles react with the alkali. Electrometric titrations of the alkali with the colloidal acid will show how far this explanation is correct.

The hydrogen ion concentration of silicic acid sol (III) at three dilutions are given in Table VI.

TABLE VI.

Dilution of sol	C_H
Pure	0·00832
1:1	0·00107
1:4	0·000219

If we plot the C_H in Table VI against the relative concentrations of silicic acid sol we get Curve IX. Curves X and XI were obtained from the work of Pallmann [1930] on electrodialysed acid clay and humic acid suspension respectively. Curves IX′, X′ and XI′ give in the same figure the variations of C_H with square root of relative concentrations. It will be seen that all these curves have a concavity towards the ordinate which show that we are neither dealing with another weak acid nor with a strong acid as ordinarily understood in classical electrochemistry. The concavity indicates an increase of the activity co-efficient with concentration instead of a decrease. The curves below thus show great divergences from the concept of weak acids as they are ordinarily understood. *The acids behave as ultrastrong acids.*

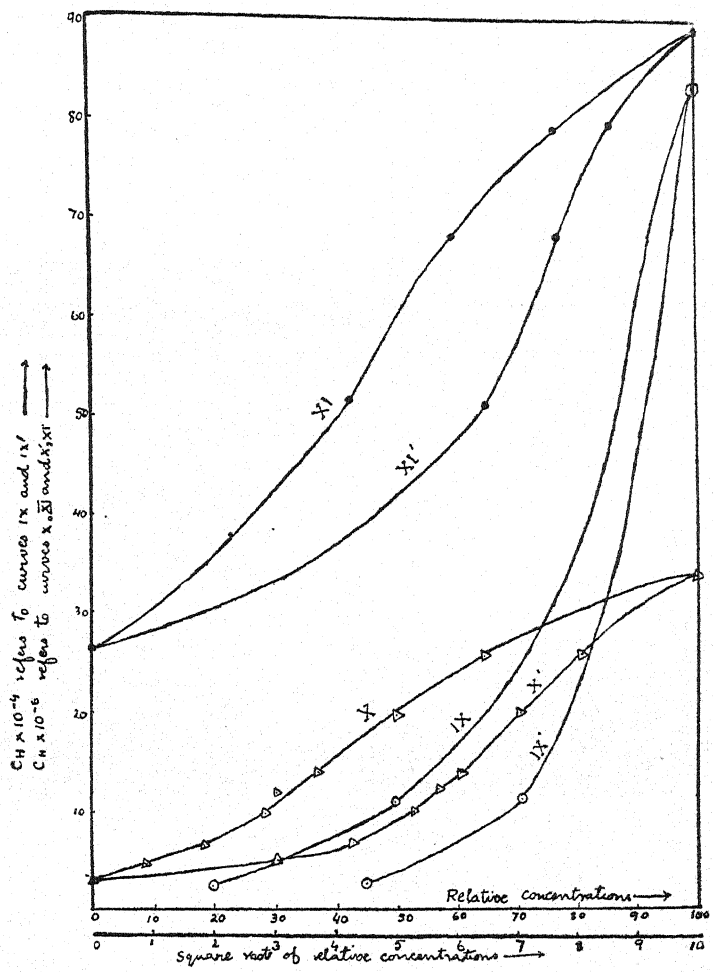


Fig. 10.

The above facts can be explained by the following tentative picture. On the surface of the colloidal particle (the primarily absorbed layer) there is a certain number of primarily absorbed ions whose valency determines the so-called 'basicity' of the colloidal acid. Around the primary layer, there is a secondary layer of H ions whose activity is determined by the measured electrode potential. As the distance between two particles is decreased by increasing the concentration, the H ions in the outer layer of one particle influence the distribution of H ions on the outer layer of other colloidal particles in such a way that the observed activity is much greater than what can be calculated from classical electrochemistry. At higher concentrations of the colloidal particles, regions of the double layer overlap in such a way as to lead to the entrainment of a part of the free hydrogen ions and thus lead to a sharp change in the curvature indicating a rapid diminution of the observed activity of the hydrogen ions. *These considerations will be taken up in detail in a subsequent paper.*

B.—Humic acid sol.

TABLE VII.

Potentiometric titrations with humic acid sol.

(Strength of baryta—0·00927 N.)

Sol I (Curve XII) Sol=75 c.c.	
Alkali	pH
c.c.	
0	5·00
0·1	5·15
0·3	5·45
0·4	5·55
0·5	5·66
0·6	5·79
0·7	5·96
0·8	6·06
1·0	6·43
1·1	6·61
1·2	6·82
...	...

TABLE VIII.

Conductometric titrations with humic aicd sol.

(Strength of baryta—0·0000927 N.)

Sol I (1:1, Curve XIII) Baryta=50 c.c.	
Sol	Sp. conductivity × 10^5
c.c.	
0	1·886
1·0	1·785
2·0	1·699
2·5	1·716
5·0	1·926
6·0	2·019
7·0	2·116
8·0	2·154
10·0	2·334
11·0	2·432
13·0	2·596
15·0	2·770

From the above Tables we find that humic acid sol does not also show any regular behaviour. There is no marked break in the curve. The conductometric titration curve shows again the same feature that in this case also we are dealing with a strong acid. This system will be taken up in detail in a subsequent paper.

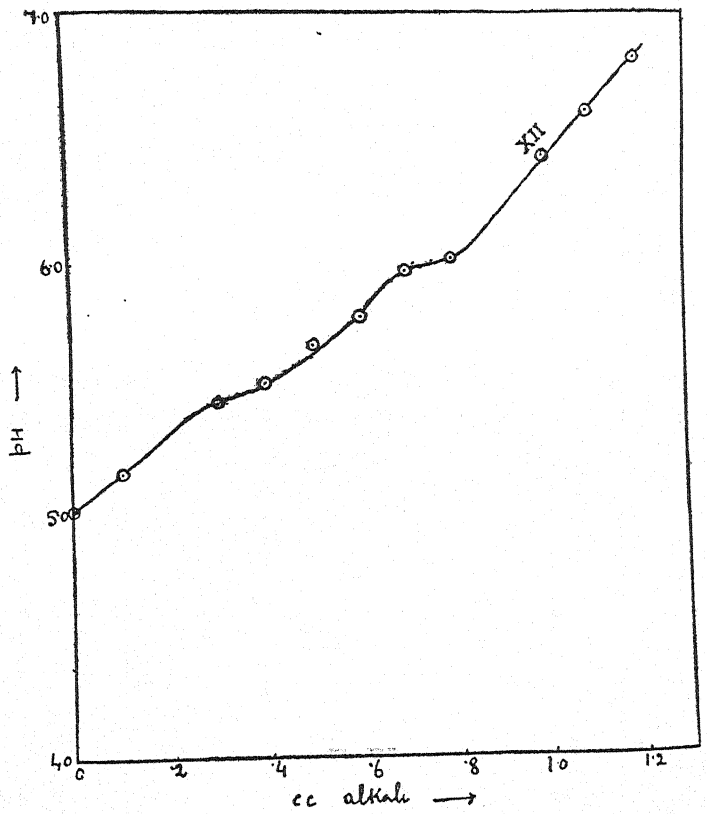


Fig. 11.

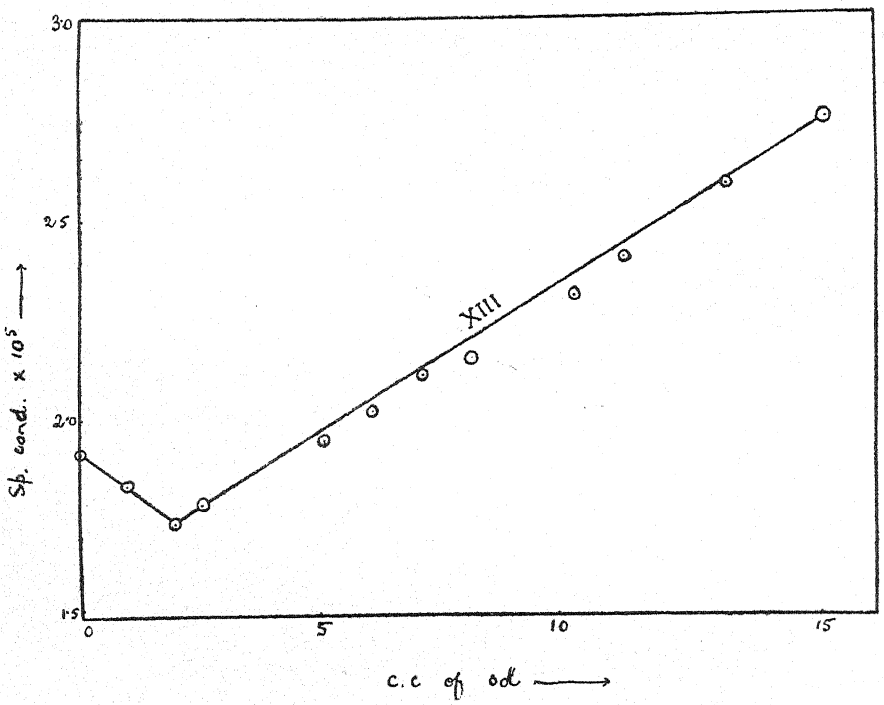


Fig. 12.

C.—Aluminium hydroxide sol.

TABLE IX.

Potentiometric titration with aluminium hydroxide sols.

(Sol=50 c.c.; strength of baryta solution=0·00732 N. The titration was done twice for observing the reproducibility.)

I (Curve XIV)		II (Curve XV)	
Alkali	pH	Alkali	pH
c.c.		c.c.	
0	6·67	0	6·63
0·1	8·07	0·1	8·01
0·2	8·53	0·2	8·44
0·3	8·53	0·3	8·43
0·5	8·52	0·5	8·41
1·0	8·54	1·0	8·49
1·5	9·56	1·2**	8·47**
2·0	9·24	...	...
2·5	9·18	...	...
3·0*	9·04*	...	...

*Coagulation was noted at this stage. It was not looked for earlier.

**After this point the sol was found to have precipitated and the e.m.f. was unsteady.

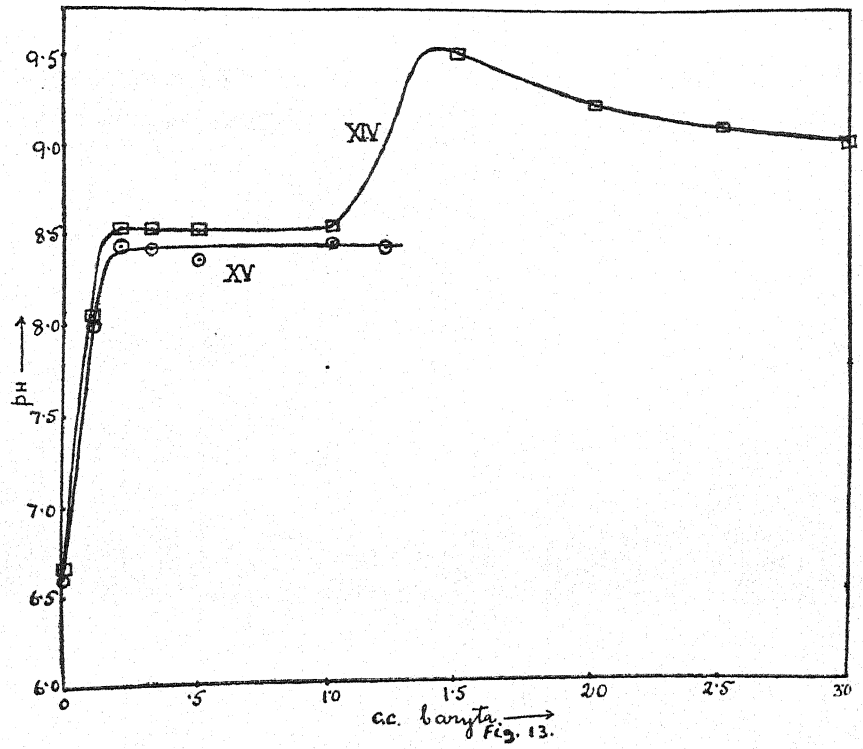


Fig. 13.

TABLE X.

(Sol=50 c.c.; strength of baryta solution=0·006213 N). The titration was continued for several days and was done twice for observing the reproducibility.

I (Curves XVI a to b)			II (Curves XVII a to b)		
Date of measurement	Alkali	pH	Date of measurement	Alkali	pH
	c.c.			c.c.	
17th February 1932 .			22nd February 1932		
(Curve XVI a) . .	0	6·68	(Curve XVII a).	0	7·18
	0·1	7·24		0·1	7·61
	0·2	7·78		0·2	8·06
	0·3	8·09		0·3	8·32
	0·4	8·86		0·4	8·72
	0·5	8·85		0·5	8·71
	0·6	8·81		0·6	8·70
18th February 1932			23rd February 1932.		
(Curve XVI b) . .	0·6 (mixture of previous day)	7·21	(Curve XVII b)	0·6 (mixture of previous day)	7 54
	0·7	7·81		0·7	8·07
	0·8	8·12		0·8	8·28
	0·9	8·53		0·9	8·58
	1·0	9·13		1·0	8·57
	1·1	9·12		1·1	8·59
	1·2	9·16		1·2	8·61
	1·3	9·32		1·3	8·87
	1·4	9·36		1·4	8·89

TABLE X—*contd.*

(Sol=50 c.c.; strength of baryta solution=0·006213 N). The titration was continued for several days and was done twice for observing the reproducibility.—contd.

I (Curves XVI c to e)			II (Curves XVII c to d)		
Date of measurement	Alkali	pH	Date of measurement	Alkali	pH
	c.c.			c.c.	
19th February 1932 (Curve XVI c) . .	1·4 (mixture of previous day)	7·30	24th February 1932. (Curve XVII c) .	1·4 (mixture of previous day)	7·96
	1·5	8·13		1·5	8·13
	1·6	8·09		1·6	8·28
	1·7	8·34		1·7	8·47
	1·8	6·77		1·8	8·57
	1·9	6·54		1·9	8·56
	2·0	6·54		2·0	8·74
	2·1	6·54		2·1	8·81
				2·2	8·96
20th February 1932 (Curve XVI d) .	2·1 (mixture of previous day)	9·07	25th February 1932. (Curve XVII d)	2·2 (mixture of previous day)	7·45
	2·2	9·22		2·3	7·68
	2·3	9·22		2·4	7·83
				2·5	7·98
				2·6	7·97
				2·7	8·03
21st February 1932 (Curve XVI e) . .	2·3 (mixture of previous day)	6·14			
	2·4	6·13			
	2·5	6·12			
	2·6	6·41			

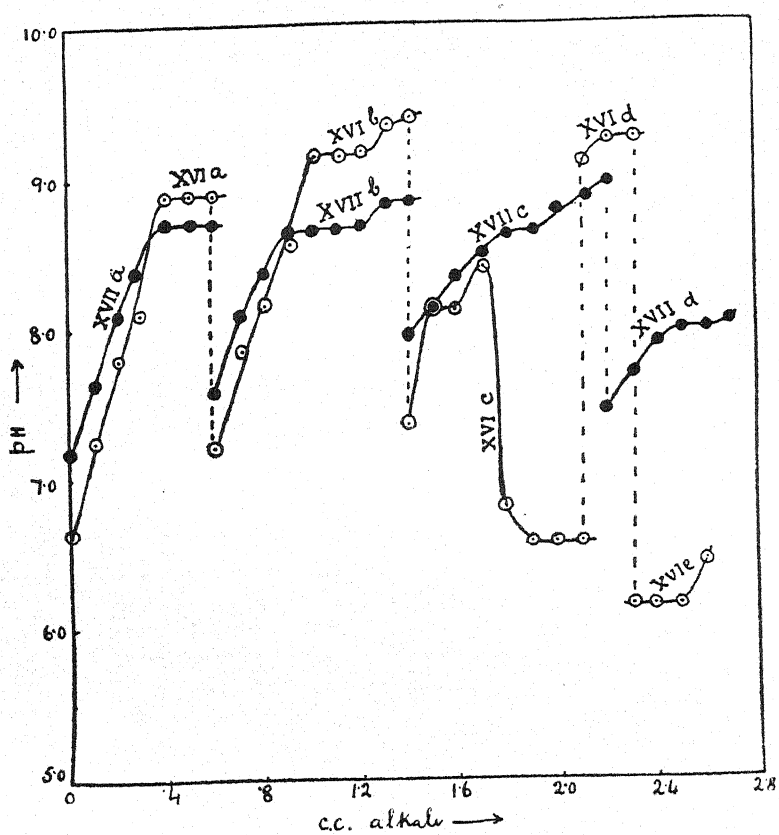

10·0
9·0
8·0
7·0
6·0
5·0
pH
0
·4
·8
1·2
1·6
2·0
2·4
2·8
c.c. alkali
XVII a
XVI b
XVII b
XVI d
XVII c
XVII d
XVI c
XVI e

Fig. 14.

TABLE XI.

Simultaneous measurement of changes in H ion, Cl ion and in sp. conductivity in the newly devised titration vessel by the gradual addition of baryta solution (0·00111 N).

(75 c.c. of aluminium hydroxide sol was taken. Half-an-hour interval was allowed as before between two successive readings so as to obtain constancy in the results.)

Baryta	pH (Curve XVIII)	$Cl' \times 10^5$ (Curve XIX)	Sp. conductivity $\times 10^5$ (Curve XX)
c.c.			
0	5·34	2·17	4·94
0·1	5·36	2·01	4·76
0·2	5·37	0·965	4·59
0·3	5·37	0·834	4·28
0·4	5·37	0·834	4·14
0·5	5·41	0·805	3·77
0·6	5·78	0·761	3·20
0·7	6·19	0·748	2·97
0·8	7·02	1·344	2·72
0·9	7·53	2·6	2·61
1·0	7·63	4·42	2·55
1·1	7·64	4·59	2·51
1·2	7·64	5·05	2·56

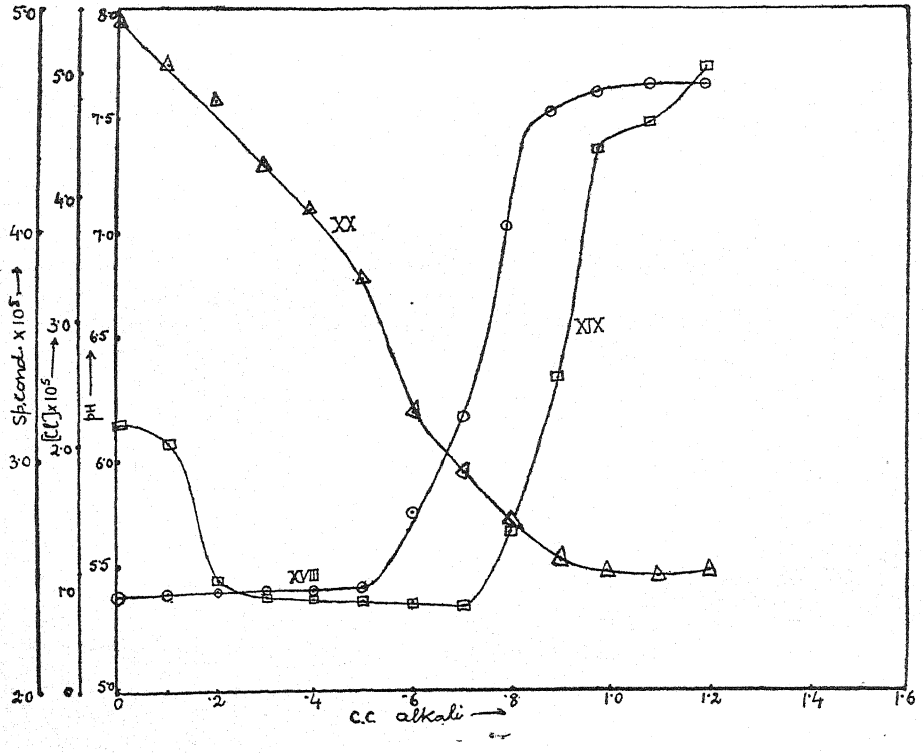


Fig. 15.

In the case of aluminium hydroxide sol, as already stated in the experimental portion, greater precaution was taken to prevent the sol from coming into contact with the oxygen or the carbon dioxide of the air. It will be found that the results in Table IX (Curves XIV and XV) are fairly reproducible. Also from the results summarised in Table X we find that Curves XVI(*a*) and XVII(*a*), which were gone through in one day and in the first day of each series, show fairly satisfactory reproducibility, but the reproducibility of the other corresponding curves (Curves XVI(*b*) and XVII(*b*), XVI(*c*) and XVII(*c*), etc.) are far from being satisfactory. Moreover, we find from Table X that on keeping a mixture of sol and baryta overnight an increase in acidity is observed on the following day. Evidently in such cases the time of interaction of the alkali with the sol is a very important factor. Moreover as the sols are allowed to lie overnight, there is an increased possibility of the access of carbon dioxide into the sol, inspite of all the precautions taken, and this is one of the reasons for not obtaining reproducible readings of two series of experiments on 2nd, 3rd and successive days (Curves XVI(*b*) and XVII(*b*), XVI(*c*) and XVII(*c*), etc.). We have determined the carbonate content of aluminium hydroxide sol and

of the sols left over after the end of the experiments (Cp. Table X). Table XII gives the results.

TABLE XII.

The results represent mean of two observations which agreed within 10 per cent.

Sample	Conc. of carbonate in normality
Original sol	0·000356
I Residual sol of Table X	0·000748
II Residual sol of Table X	0·000508

It would be apparent that with the precautions taken (older apparatus) the access of carbon dioxide into the system during the experiment could not be excluded.

The initial portion here also resembles the neutralisation of a strong acid.

The results given in Table XI were taken with the apparatus with sealed in ground glass, joints. The hydrogen ion and chlorine ion concentrations and the specific conductivities were taken simultaneously with a much greater precaution to exclude oxygen or carbon dioxide. This sample of aluminium hydroxide sol was stocked in an arrangement in which the access of these gases was carefully avoided. The results obtained show greater concordance. Curve XVIII shows that the sample of aluminium hydroxide sol has the character of a strong acid. The end point of titration with baryta solution was approximately the same both with the potentiometric and conductometric titration curves (Curves XVIII and XX). The curve representing the variation in Cl ion (Curve XIX) is complicated but shows a minimum at approximately the same region, namely the end point of the titration (0·7 c.c. of the baryta solution). The titration of aluminium hydroxide sols will be taken up fully in a subsequent paper in the light of the knowledge gained in this and the previous papers.

The results obtained in the present paper emphasise the need of great caution in accepting the conclusions often drawn from experiments with sols as also from titrations of acid clay and soil. The present work suggests that adequate attention should be paid to the exclusion of oxygen and carbon dioxide, and to the special aspects arising out of interactions involving interfaces. The results with silicic acid sol and humic acid sol show that characteristic interactions involving interfaces are

present and that ordinary preparations of colloidal solutions of common acidic substances show peculiarities which are not contemplated in the classical electrochemistry of molecular solutions of acids. The approximate values of the dissociation constants of a weak acid, like silicic acid, the variation in its hydrogen ion concentration with the concentration of the sol and the forms of the conductometric titration curves show the necessity for a departure from the usual electrochemical notions and for a different theoretical treatment on the lines outlined by one of us [Mukherjee, 1922, 1925, 1929].

Summary.

1. It has been shown that colloidal solutions of silicic acid behave in many respects as a strong acid and, as far as present evidence indicates, in some respects even as an acid stronger than the so-called strong acids. In some other respects it has the character of a weak acid. Humic acid (colloidal) also shows in some respects the character of a strong acid.

2. Experimental evidence has been given showing the need for accurate technique and exclusion of carbon dioxide in such measurements.

3. The peculiarities of interactions involving interfaces are brought out.

4. The role of small amounts of electrolytes is shown to be considerable in determining the form of the titration curve of an aluminium hydroxide sol.

5. Arrangements devised for further work, which exclude carbon dioxide and oxygen and also allow simultaneous measurements necessary on theoretical grounds in such cases, but which have been so far overlooked in work with colloidal clay and soil, have been described.

REFERENCES.

Mukherjee, J. N. (1922). *Phil. Mag.* **44,** 321.
——, ——— (1925). *J. Ind. Chem. Soc.* **2,** 191.
——————— (1929, 1). Proc. Indian Science Congress, p. 93.
——————— (1929, 2). *Kolloid Zeitschr.* **49,** 362.
Mukherjee, J. N. and Sen, H. K. (1931). *Ind. J. Agric. Sci.* **1,** 189.
Mukherjee, J. N., Roychoudhury, S. and Biswas, M. (1931). *J. Ind. Chem. Soc.* **8,** 373.
Muttoné, F. and Pauli, Wo. (1931). *Kolloid Zeitschr.* **57,** 312.
Noyes A. A. and Ellis, J. H. (1917). *J. Amer. Chem. Soc.* **39,** 2532.
Pallmann, H. (1930). *Koll. Chem. Beihefte* **30,** 334.
Rabinowitsch, A. J. and Kargin, V. A. (1928). *Z. Phys. Chem.* **133,** 203.
Rabinowitsch, A. J. and Laskin, E. (1928). *Z. Phys. Chem.* **134,** 387.
Randall, M. and Young, L. E. (1928). *J. Amer. Chem. Soc.* **50** 995.
Wassilew, P. S. and Rabinowitsch, A. J. (1931). *Kolloid Zeitschr.* **56,** 305 .
Weiser, H. B. (1931). *J. Phys. Chem.* **35,** 1388.
Wintgen, R, and Biltz, M. (1923). *Z. Phys. Chem.* **107,** 403.
Wintgen, R. and Kühn, O. (1928). *Z. Phys. Chem.* **138,** 135.

PHYSIOLOGIC SPECIALISATION IN *SCLEROSPORA GRAMINICOLA* (SACC.) SCHROET.

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(With Plate LXXI.)

Sclerospora graminicola (Sacc.) Schroet. has been studied extensively in recent years. It has been recorded chiefly on the species of Setaria from many parts of the world and also on *Pennisetum typhoideum* L. in India. The occurrence of physiologic specialisation in this parasite has not been clearly demonstrated, although suggestions have been made from time to time that this fungus does not pass from one host to another under field conditions. This view could not, however, be tested experimentally since the conditions under which oospores of the fungus can infect its hosts were not fully determined. The recent work of Melhus *et al.* [1928], of Uppal and Kamat [1928], and of Hiura [1929] in inoculating successfully the hosts of *Scl. graminicola* with its oospores, has made it possible for this study to be made. The results of this investigation, a summary of which has previously been reported [Uppal and Desai, 1931], are presented in the following paper.

COMPARATIVE STUDY OF *Sclerospora graminicola* ON DIFFERENT HOSTS.

Morphological characteristics.

The life-cycle of *Sclerospora graminicola* on all its hosts comprises a conidial as well as an oosporic phase. The material of the conidial phase of the Sclerospora on

Pennisetum was collected from the leaves of infected plants at Poona during the period of optimum conidiophore production from 3 to 4 A.M., and similar material of the fungus on *Setaria italica* (L.) Beauv. was secured from Madras through the courtesy of Mr. S. Sundararaman. The Madras material was killed in 70 per cent. alcohol with a trace of formaldehyde; but it was so badly plasmolysed that it was not in a good condition for study. The conidial material from Pennisetum only was therefore compared with the material of the conidial phase of *Sclerospora graminicola* on *Setaria viridis* (L.) Beauv. and *S. magna* Griseb., previously studied by Weston [1924] and Weston and Weber [1928] in the United States.

The material of the resting-spore phase of the fungus on *Setaria viridis*, *S. magna* and *S. italica* was kindly furnished by Dr. I. E. Melhus, Dr. George F. Weber and Mr. S. Sundararaman, respectively. Similar material on Pennisetum was collected at Poona.

The conidial stage.—An excellent description of the structural development of the conidiophore of *Sclerospora graminicola* on *Setaria viridis* has been given by Weston [1924]. He says, "In *Sclerospora graminicola*, on the contrary, one of the primary branches stands out more or less obviously as a continuation of the main axis both in direction and in extent of growth. From the continuation of the main axis other main and secondary branches grow out at irregular intervals, usually at angles of 45° to 90°. As a result, the conidia at the ends of the branches lie more frequently in irregularly disposed groups than in an approximate hemisphere," as is the case in *Sclerospora sorghi* (Kulk.) Weston and Uppal, and the conidial Sclerosporas of the Orient. The description becomes very vivid when the illustrations of the well-developed conidiophores of the Sclerospora on Pennisetum are examined (Plate LXXI, A1-4).

The sterigmata of *Scl. graminicola* on *Setara magna* and *S. viridis* (cf. Weston and Weber, 1928, Fig. 4, B; Weston, 1924, Plate 2, N, T, V, W) agree in shape and arrangement with those of the fungus on Pennisetum (Plate LXXI, A1, 3, 4). In length, however, they show great variation on all the hosts, usually varying from 3·3 to 13·3 μ, the average length being about 7 μ.

The average length of the conidiophores of *Scl. graminicola* on different hosts is not as variable a feature as Melhus *et al.* [1928] considered. Although they show a wide range in individual variation from about 190 μ to 370 μ as shown in the following two sets of measurements, the average length of conidiophores developed on different hosts is about the same. Melhus *et al.* [1928] have given the average length of the conidiophores of the fungus on *Setaria viridis* as 267·8 μ. while the conidiophores developed on Pennisetum average 256 8 μ in length. Weston [1924], however, has found the conidiophores of the Sclerospora on *Setaria viridis* to vary from 100 μ to 200 μ, while Butler [1907] gives as low a figures as 100 μ for the

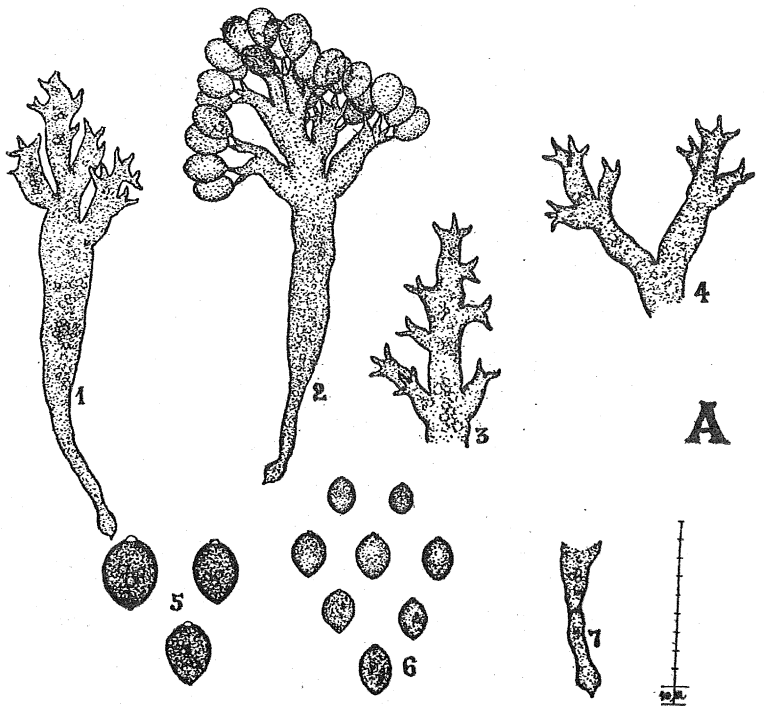


Fig. 1.

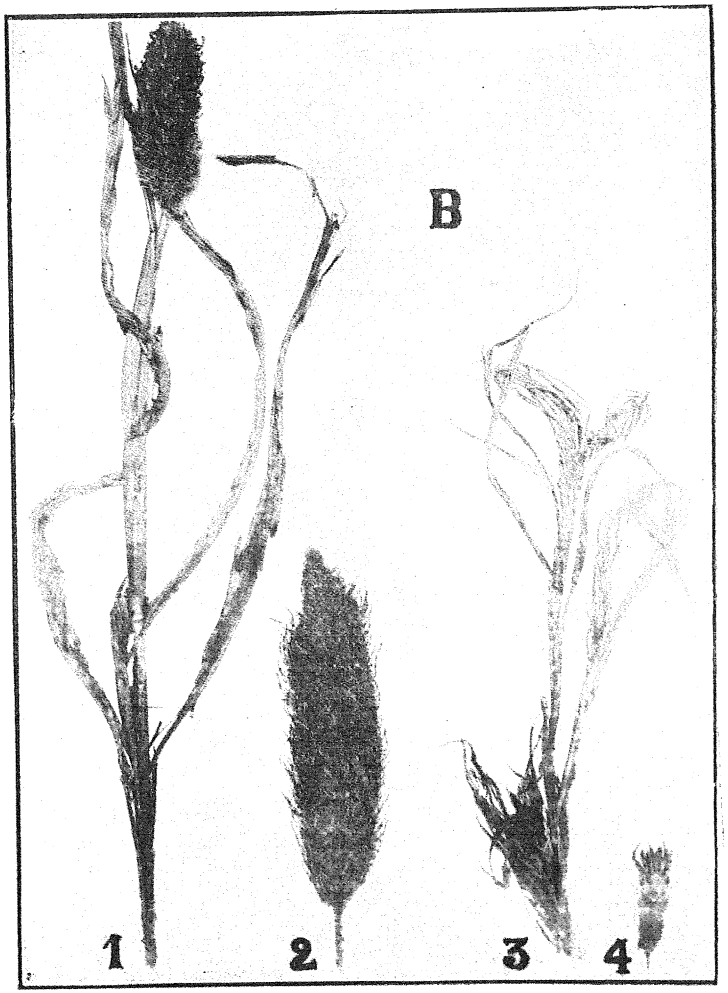
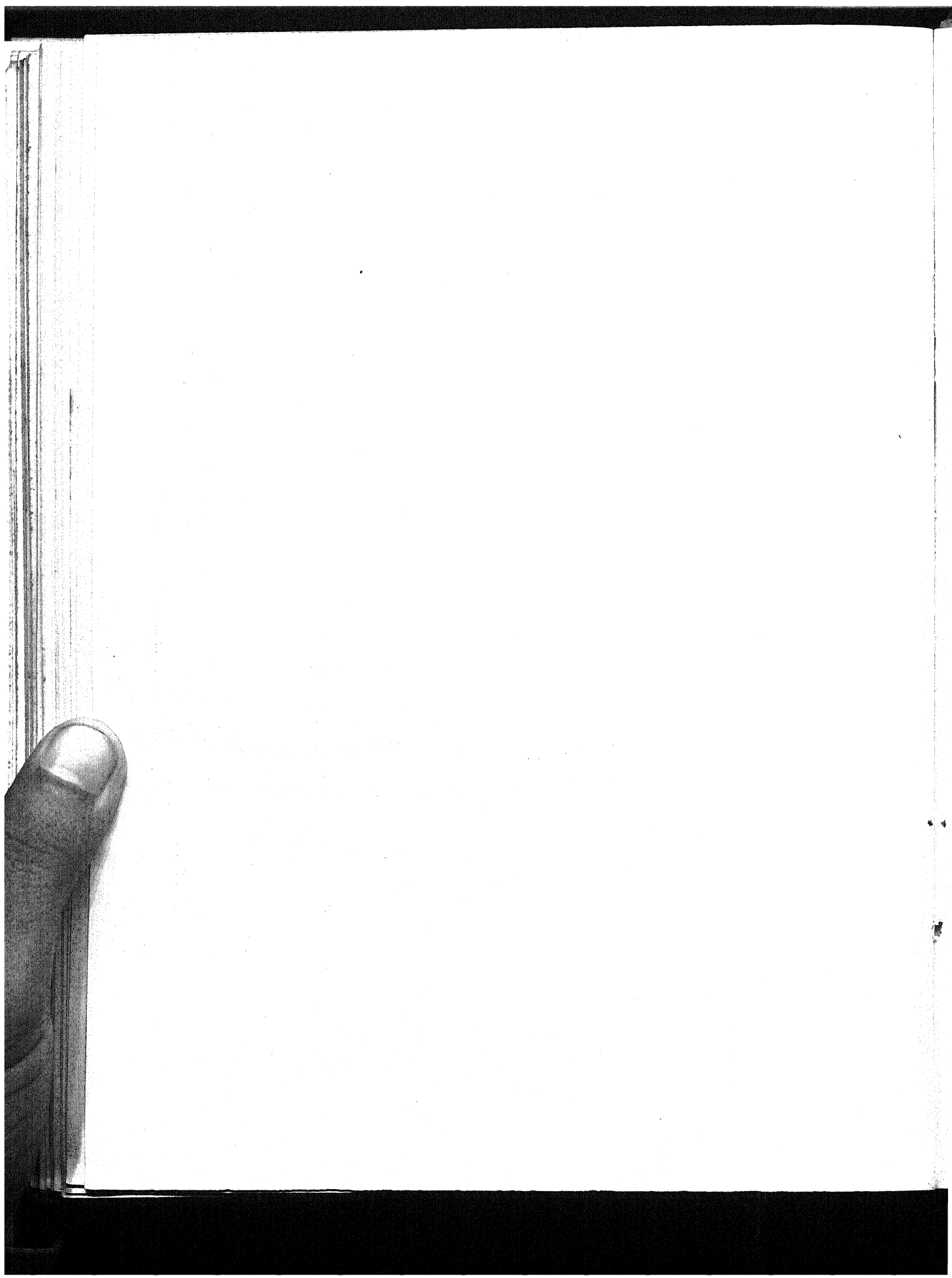


Fig. 2.

PLATE LXXI.

(*For explanation please see p. 678.*)



fungus on Pennisetum. It appears therefore that these two writers did not use luxuriantly sporulating material, since the figures on the average length of conidiophores given by Melhus *et al.* [1928] and the present writers differ markedly from the corresponding figures given by Weston and Butler.

Length of conidiophores from *Setaria viridis**	Length of conidiophores from *Pennisetum typhoideum*
375·3μ	193·3μ
234·0μ	226·6μ
214·5μ	283·3μ
288·6μ	200·0μ
241·8μ	293·3μ
319·8μ	326·6μ
234·0μ	200·0μ
241·8μ	266·6μ
273·0μ	350·0μ
308·1μ	216·6μ
214·5μ	253·3μ
	243·3μ
11 | 2945·4μ	226·6μ
	293·3μ
267·8μ Average length.	300·0μ
	15 | 3852·8μ
	256·8μ Average length.

There is, however, another feature of the conidiophores of *Scl. graminicola*, which, though it marks off this fungus as a distinct species from such forms as *Scl. sorghi*, yet has a distinctive value in that it occurs with extreme regularity. This feature is that the conidiophores of the Sclerospora on Pennisetum and the species of Setaria are, as a rule, non-septate, only in very rare cases a cross wall being developed cutting off a basal cell (Plate LXXI, A 7; also see Weston, 1924, Plate 2, X, Y; Weston and Weber, 1928, Fig. 4, J).

* These data are those reported by Melhus, Van Haltern and Bliss [1928].

TABLE I.

Comparative measurements of conidia of Sclerospora graminicola on Pennisetum typhoideum, Setaria magna and S. viridis.

Length				Diameter			
	Living material from		Preserved material from		Living material from		Preserved material from
Classes in μ	No. of conidia in 400	No. of conidia in 250	No. of conidia in 400	Classes in μ	No. of conidia in 400	No. of conidia in 250	No. of conidia in 400
	Pennisetum typhoideum	*Setaria magna**	*Setaria viridis**		*Pennisetum typhoideum*	*Setaria magna**	*Setaria viridis**
11 to 12·9 . .	0	0	7	9 to 10·9 .	0	0	1
13 to 14·9 . .	3	2	25	11 to 12·9 .	1	4	111
15 to 16·9 . .	15	11	67	13 to 14·9 .	21	39	162
17 to 18·9 . .	63	35	119	15 to 16·9 .	243	105	92
19 to 20·9 . .	179	59	89	17 to 18·9 .	82	72	30
21 to 22·9 . .	75	69	51	19 to 20·9 .	48	22	4
23 to 24·9 . .	47	46	22	21 to 22·9 .	5	7	0
25 to 26·9 . .	14	17	10	23 to 24·9 .	0	1	0
27 to 28·9 . .	2	6	6				
29 to 30·9 . .	1	4	1				
31 to 32·9 . .	0	0	1				
33 to 34·9 . .	1	0	2				
35 to 36·9 . .	0	1	0				

* The date on the measurement of conidia of *Sclerospora graminicola* on *Setaria magna* and *S. viridis* are those given by Weston and Weber [1928] and by Weston [1924], respectively.

The conidia of *Sclerospora graminicola* developed on different hosts resemble each other in general characteristics. From the data presented in Table I, it will be seen that the measurements of the fresh, living spores of the fungus on the three hosts agree very closely. The modes of length of the conidia of the Sclerospora on Pennisetum and *Setaria magna* fall in the 19 to 21 μ (or 17 to 19 μ for preserved

material) and 21 to 23μ classes, respectively. The mode of diameter of the conidia is the same, 15 to 17μ class ; but in the preserved material from *Setaria viridis*, the class value is decreased by 2μ, *i.e.*, 13 to 15 μ. However, as suggested by Weston [1924], if the modes of length and of diameter of the preserved conidia are increased by one 2 μ class, the agreement between the preserved material from *Setaria viridis* and the living material from Pennisetum and *Setaria magna* becomes very close. There is thus relatively little difference in the size of conidia produced on different hosts, and it appears that this characteristic persists unaltered when the fungus develops on Pennisetum under sub-tropical conditions.

Several large conidia were also encountered on the luxuriantly sporulating leaves of Pennisetum collected at night, and these measured from 38 to 43μ× 20·8 to 25 μ (Plate LXXI, A 5). Similar bodies were also found by Melhus *et al.* [1928] in the conidial material from *Setaria viridis*, their measurements being 43μ×18·6μ. It is thus permissible to conclude that Shirai [1897], while reporting the size of giant conidia as 38·4 to 57·6μ×19·2 to 24μ, was probably dealing with the conidial phase of *Scl. graminicola* and not with that of any other species, as was Weston [1924] led to suspect.

Although there is considerable individual variation in shape (Plate LXXI, A 6 : see also Weston, 1924, Plate 2, M ; Weston and Weber, 1928, Fig. 4, K), the conidia are usually broadly elliptical to rounded cylindric. They also show an apical papilla of dehiscence, which at germination gelatinizes, leaving a pore through which zoospores emerge. The germination of conidia is invariably by the formation of zoospores, although Weston and Weber [1924] have observed a few cases in *Scl. graminicola* on *Setaria magna*, where the spores germinated by short abortive hyphae. The direct germination of conidia has not, however, been observed in the case of the Sclerospora on Pennisetum. The optimum temperature of sporulation of the Sclerospora on Pennisetum was 16 to 18°C., and the conidia germinated profusely at temperatures between 18 and 23°C. The figures on germination show close agreement with those reported by Weston and Weber [1928] for the fungus on *Setaria magna*.

The oosporic stage.—While agreeing in the general characteristics of the oogonial phase, the fungus on Pennisetum produces symptoms on this host, which readily distinguish it under field conditions. The oosporic phase on Pennisetum, which develops at a later stage in the growth of the plant, differs markedly in that the spores which are formed in the leaf tissue as brown dots do not cause the extensive destruction of the mesophyll (Plate LXXI, B 1) with the consequent shredding of leaves into masses of tangled fibres characteristic of *Scl. graminicola* on Setaria (Plate LXXI, B 3 ; see also Weston and Weber, 1928, Plate 2C) and of *Scl. sorghi*. In Pennisetum, however, only the leaves below the ears split into shreds. This

absence of shredding of the leaves of Pennisetum is probably due to the scattered arrangement of the oospores in the leaf tissue, while in Setaria these spores are arranged in linear rows, along which splitting takes place. The head is also affected and, as a result of this attack, exhibits teratological malformations of its parts. The shredding of the floral parts is characteristic of the fungus on Setaria (Plate LXXI, B 4) but not on Pennisetum (Plate LXXI, B 2).

TABLE II.

Comparative measuremenis of oospores of Sclerospora graminicola *on* Pennisetum typhoideum, Setaria, italica, S. viridis *and* S. magna.

Classes in	Number of oospores in 400			Number of oospores in 350
	Pennisetum typhoideum	*Setaria italica*	*Setaria viridis*	*Setaria magna**
21 to 22·9	0	0	0	1
23 to 24·9	0	0	0	5
25 to 26·9	5	3	3	7
27 to 28·9	10	3	8	10
29 to 30·9	52	40	36	37
31 to 32·9	56	43	52	59
33 to 34·9	103	94	77	58
35 to 36·9	115	131	143	68
37 to 38·9	31	41	35	55
39 to 40·9	22	37	33	36
41 to 42·9	2	7	9	9
43 to 44·9	2	1	3	5
45 to 46·9	2	0	1	0

*The data on the measurement of oospores of *Sclerospora graminicola* on *Setaria mayna* are those given by Weston and Weber [1928].

In shape and size the oospores of the fungus on different hosts show very close resemblance. They are spherical in shape, and their size also is very uniform, the majority (more than 75 per cent.) falling in the class between 30 to 36μ, the mode being 35 to 36·9μ (Table II). The single oospore within the oogonium is surrounded by a wall varying in thickness but most frequently 1·9 to 2·9μ (Table III), and

ranging from pale amber of Ridgway's " Colour Standards " to a faintly golden colour.

TABLE III.

Comparative measurements of wall width of oospores of Sclerospora graminicola *on* Pennisetum typhoideum, Setaria italica, S. viridis *and* S. magna.

Classes in μ	Number of oospores in 150			
	Pennisetum typhoideum	*Setaria italica*	*Setaria viridis*	*Setaria magna*
0·3 to 1·09	18	15	4	8
1·1 to 1·89	50	38	37	46
1·9 to 2·69	66	72	84	71
2·7 to 3·49	14	22	24	20
3·5 to 4·29	2	3	1	5

Physiological distinctions.

From the foregoing statement it will be seen that, except for some minor symptoms which this fungus produces on Pennisetum the Sclerospora on this host closely resembles the downy mildew on Setaria both in its oosporic as well as conidial phase. It was therefore of interest to study the host range of the fungus and also to determine whether there was any physiological difference in the forms found on the different host plants.

As already noted, the greatest difficulty encountered in cross inoculations with *Scl. graminicola* was the failure of its oospores to germinate and cause infection of its hosts under controlled conditions, since it is very difficult to produce infection with the living conidia. This necessitated a large number of trials to determine the conditions under which infection takes place in *bajri* (*Pennisetum typhoideum*). The results of these experiments have previously been reported, and show that it is possible to obtain artificial infection of this host by placing finely powdered oospore material on the seed in the soil [Uppal and Kamat, 1928]. Recently Chaudhuri [1932] has also reported successful inoculation of *bajri* with oospores ; but it is surprising that this writer has made no mention whatsoever of similar results previously reported by Uppal and Kamat [1928].

The host plants used in these studies were *Pennisetum typhoideum*, *Setaria italica**, *S. magna** and *Euchlaena mexicana* Schrad., and the oospore materials

* The seed of *Setaria italica* and *S. magna* was kindly supplied by Mr. S. Sundararaman and Dr. George F. Weber, respectively.

from all these hosts (except the latter) and also from *Setaria viridis* were used in cross inoculations. The seed of these hosts was sown in 6-inch pots filled with sterilised river soil. The seed-bed was watered in every case before the seed was sown. In each bed furrows were opened by a sterile knife, and the seed, which was moistened and rolled in finely powdered oospore material, was sown in the furrows. Enough oospore material was again placed on each seed before the furrows were closed with the soil. In all cases water was supplied by capillarity. In cross inoculation experiments two controls were always provided : in one case the oospore material of the fungus was used to inoculate its host, but no inoculum was used in the other case. The results are summarised in Table IV.

TABLE IV.

Results obtained by exposing four species of Poaceae to infection by oospores of Sclerospora graminicola *collected from four of its hosts.*

Species exposed to infection	Per cent. plants infected*			
	Oospores from			
	Setaria viridis	*Setaria magna*	*Setaria italica*	*Pennisetum typhoideum*
Setaria italica	70	77·1	83·3	0·0
	55	74·0	43·7	0·0
	26·1	18·1	42·8	0·0
Setaria magna	14·8	47·8	10·2	..
	48·1	42·8	21·8	..
	...	45·0	...	0·0
Euchlaena mexicana	71·0	40·0	14·0	0·0
Pennisetum typhoideum	0·0	0·0	0·0	60·0
	0·0	0·0	0·0	53·8
	0·0	0·0	0·0	75·0
Setaria glauca	0·0	0·0	0·0	0·0
Panicum miliaceum	0·0	0·0	0·0	0·0

*The total number of plants exposed to infection varied from 28 to 328 in each case.

Cross inoculation of Setaria italica *with oospore material from* S. viridis, S. magna *and* Pennisetum typhoideum.—On June 27, 1930, seedlings of *Setaria italica* were exposed to infection by the germinating oospores from *S. viridis*. By July 7, most of the plants showed distinct mottled areas on the first leaf, and three days later there was scanty sporulation noticeable in a few cases. In another three days there was abundant conidial fructification on the pallid areas of the leaves. It was also observed that infection usually started at the base of the first leaf and gradually spread to other leaves as they developed.

The above experiment was repeated twice with essentially similar results. In one case, however, the seedlings showed the first signs of the disease five days after they were exposed to infection. In all cases control plants which were not inoculated remained healthy.

In another series of experiments (July 10) seedlings of *Setaria italica* were exposed to infection by oospores from *S. magna*. The symptoms developed within six days, and the leaves were soon covered with the conidial sporulation. These experiments were repeated in August 1930, and the results obtained were the same. The controls remained healthy.

Negative results were obtained when oospore material from *Pennisetum typhoideum* was used to inoculate the seedlings of *Setaria italica*. The control seedlings, however, gave abundant infection when oospores from *S. italica* were used as inoculum.

Cross inoculation of Setaria magna *with oospore material from* S. viridis, S. italica *and* Pennisetum typhoideum.—On July 12, 1930, experiments similar to those described above were made with *Setaria magna*. The seed germinated poorly, and the seedlings were slow and stunted in growth. Nine days after exposure to infection by the oospore material from *S. viridis*, two seedlings showed pale yellow blotching on the first leaf. Four days later another two seedlings developed similar symptoms. The percentage of infection, however, was low. The experiments were repeated with essentially similar results.

When oospore material from *S. italica* was used to inoculate seedlings of *S. magna* (July 29), infection was noticeable 13 days after exposure. About 10 per cent. of seedlings supported conidial fructification on their leaves. The experiments were repeated with similar results.

Experiments in which oospore material from Pennisetum was used gave negative results.

Cross inoculation of Euchlaena mexicana *with oospore material from* Setaria viridis, S. magna, S. italica *and* Pennisetum typhoideum.—In all cases when teosinte seedlings were inoculated with the oospore material from Setaria, infection was readily obtained and the symptoms developed within 8-10 days. In a majority of seed-

lings pallid areas were seen on the first leaf. In no case, however, did teosinte seedlings become diseased when oospores from Pennisetum were used to inoculate.

Cross inoculation of Pennisetum typhoideum *with oospore material from* Setaria viridis, S. magna *and* S. italica.—Consistently negative results were obtained when Pennisetum seedlings were inoculated with the oospore material from Setaria. In one test oospore inoculum from Setaria was placed in the soil 24 hours before Pennisetum seed was sown; but this also gave negative results, although control seedlings inoculated with oospores from Pennisetum showed 60 per cent. infection.

In another experiment seedlings were inoculated by the method described by Melhus *et al.* [1928]. The seed of *Pennisetum typhoideum* was covered with oospores and placed on cotton kept moist by leading the wicks into distilled water below through holes made in the bottom of the test tubes. The seedlings were exposed to infection for 6-11 days, and at the end of this period were transplanted in large pots. The plants were kept under close observation throughout the period of growth; but in no case did any symptoms of the disease develop.

Cross inoculaiton of Setaria glauca *and* Panicum miliaceum *with oospore material from* Setaria viridis, S. magna, S. italica *and* Pennisetum typhoideum.—In all cases when *Setaria glauca* (L.) Beauv. and *Panicum miliaceum* L. were exposed to infection under artificial conditions, the symptoms of the disease did not develop.

Discussion and conclusions.

The experimental evidence summarised in this paper shows clearly that there are at least two physiologic forms of *Sclerospora graminicola*, which are differentiated by their pathogenic capabilities on their hosts. One form attacks *Pennisetum typhoideum* only, while the other form can infect *Setaria viridis*, *S. italica*, *S. magna* and *Euchalaena mexicana*. The two forms also differ somewhat in the symptoms which they produce on their respective hosts under field conditions. The conidial stage is very well developed in the form on Pennisetum in Bombay, a characteristic which makes it resemble the conidial Sclerosporas of the Orient. The form on Setaria, however, has an evanescent conidial phase, so that luxuriant sporulation is not generally obtained under field conditions. In both the forms the oogonial phase is predominant; but it differs greatly in the effect which it produces on the respective hosts. In Setaria the oospores in the leaf tissue cause extensive destruction of the tissue between the bundles with the consequent shredding of leaves into tangled fibres. This effect, however, is never produced in Pennisetum, in which case only the leaves enclosing the ears split into shreds. The shredding of the leaves is, indeed, such a distinctive feature of the downy mildews of Setaria and Sorghum that its absence in Pennisetum is one of the chief distinguishing features of the *bajri* mildew in the Bombay Presidency.

In 1918 it was first observed by Butler [1928] that in Pusa the Sclerospora on *Setaria italica* did not attack *Pennisetum typhoideum* grown in the vicinity. Later, Weston and Weber [1928] reported that the downy mildew on *Setaria magna* did not pass to *Pennisetum typhoideum* under field conditions in Florida. In their extensive inoculation studies Melhus *et al.* [1928] have shown that the oospores from *Setaria viridis* did not infect *Pennisetum typhoideum*. These observations therefore substantiate the experimental data recorded in this paper and suggest that the physiologic form on the species of Setaria cannot affect *Pennisetum typhoideum* grown under varying geographical conditions.

The present studies have also shown that in all cases when *Setaria glauca* and *Panicum miliaceum* were exposed to infection by the oospores from *Setaria viridis*, *S. italica*, *S. magna* and *Pennisetum typhoideum*, no symptoms of the disease developed. The failure of the two forms on Pennisetum and Setaria to transfer to *Panicum miliaceum* and *Setaria glauca* strongly suggests the presence of one or two more physiologic forms, which attack these two hosts. It was, indeed, surprising that the form on Setaria failed to pass to *Setaria glauca* when exposed under artificial conditions.

Summary.

1. *Sclerospora graminicola* is a group species consisting of at least two physiologic forms, which resemble each other very closely in the morphological characters of the conidial as well as oosporic phase.

2. Experimental evidence shows that one physiologic form attacks *Pennisetum typhoideum* only, while the other form can infect *Setaria viridis*, *S. magna*, *S. italica* and *Euchlaena mexicana*. These forms can be differentiated by their parasitic behaviour, and also by the symptoms which they produce on their hosts. The form on Pennisetum has only been recorded in India, while the form on Setaria is widespread in occurrence.

3. The form on Setaria causes extensive shredding of the leaves of its hosts; but this symptom is lacking in Pennisetum, in which case only the leaves below the ear split into shreds. Both the forms exhibit teratological malformations of the floral organs; but only the form on Setaria can cause shredding of these parts.

4. The experimental data indicate strongly the presenee of one or two more physiologic forms, which may attack *Panicum miliaceum* and *Setaria glauca*.

REFERENCES.

Butler, E. J. (1907). *Mem. Dept. Agric. Ind., Bot. Ser.* **2**, 1-24.

———— (1918). Fungi and Disease in Plants. 547 pp., illus. Calcutta and Simla.

Chaudhuri, H. (1932). *Phytopath.* **22**, 241-246.

Hiura, M. (1929). *Trans. Sapporo Nat. Hist. Soc.* **10,** 146-156.
Melhus, I. E., Van Haltern, F. H. and Bliss, D. E. (1928). *Iowa Agric. Expt. Stat. Res. Bul.* **111,** 297-340.
Shirai, M. (1897). *Bot. Mag. Tokyo* **11,** 25-29.
Uppal, B. N. and Kamat, M. N. (1928). *Agric. J. Ind.* **23,** 309-310.
Uppal, B. N. and Desai, M. K. (1931). *Phytopath.* **21,** 337-338.
Weston, W. H. (Jr.) (1924). *J. Agric. Res.* **27,** 771-784.
Weston, W. H. (Jr.) and Weber, G. F. (1928). J. *Agric. Res.* **36,** 935-963.

Explanation to Plate LXXI.

A 1. A conidiophore with the completed branch system showing sterigmata on which conidia will bud out. Note the continuation of the main axis. × 258.

A 2. A well-developed conidiophore with mature conidia showing several branches coming off almost at the same point as the main axis. The continuation of the main axis is easily discernible. This type of branching is occasionally met with. × 258.

A 3. A type of branch system in which the side branches come off at more or less regular intervals and the continuation of the main axis is very prominent. This type of branching is characteristic of *Sclerospora graminicola*. × 258.

A 4. Portion of a conidiophore cymosely branched. This type of branching is rarely encountered. × 258.

A 5. Large conidia occasionally encountered. × 258.

A 6. Conidia showing the range of shapes and sizes normally encountered. × 258.

A 7. Base of conidiophore with a basal cell rarely encountered in *Sclerospora graminicola*. × 258.

B 1. A very dry specimen of *Pennisetum typhoideum* infected with *Sclerospora graminicola*. Note that the leaves are not shredded, although they are full of oospores.

B 2 Infected earhead of *bajri* showing teratology of floral parts.

B 3. Specimen of *Setaria italica* infected with *Sclerospora graminicola* showing the shredding of the leaves into masses of tangled fibres containing oospores.

B 4. Infected earhead of *Setaria italica* showing shredding of leafy growth in the place of the normal parts of flowers.

STATISTICAL NOTES FOR AGRICULTURAL WORKERS*

No. 3†.—AUXILIARY TABLES FOR FISHER'S Z-TEST IN ANALYSIS OF VARIANCE.

BY

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The interpretation of results of field experiments designed on the model of Fisher's "Latin Square" or "Randomized Block" depends ultimately on his z-test for the significance of the difference of two variances. Let $v_1=s_1^2$, and $v_2=s_2^2$ be two variances based on n_1 and n_2 degrees of freedom $(v_1 > v_2)$. Then "z" is defined by the equation

$$z = \tfrac{1}{2} \log_e \left(\frac{v_1}{v_2}\right) \quad . \quad . \quad . \quad . \quad . \quad (1)$$

The 5 per cent. and one per cent. points for the distribution of "z" are given in Fisher's Table VI (Statistical Methods for Research Workers, 1930, pp. 212—215).

It is, of course, absolutely necessary to use natural logarithms (*i.e.*, to the base "e") in connexion with Fisher's Table VI. With suitable tables of natural logarithms the procedure would be quite simple and straightforward. But such tables are not always available, and usually the common logarithms (to the base "10") have to be converted into natural logarithms. This is apt to cause trouble to field

* We are receiving a large number of enquiries of a statistical nature from agricultural workers in different parts of India. Many of these enquiries are of considerable general interest, and it is proposed to publish notes on selected topics from time to time. These notes will deal mainly with statistical methods and procedure, and it is not intended that they should always contain new matter—Ed.

† Nos. 1 and 2 of this series appeared in this Journal, Vol. II, Parts I and II respectively.

workers not familiar with the use of natural logarithms. In fact, I have often been asked whether something could not be done to eliminate the necessity of using natural logarithms.

For ordinary routine analysis of field trial data, it is possible to construct auxiliary tables with the help of which the z-test can be applied without recourse to natural logarithms. I am giving here six such tables, two for working with ordinary logarithms (to the base 10), two for working directly with the ratio of standard deviations and two for the ratio of variances, one table in each set corresponding to the 5 per cent. and the other to the 1 per cent. points in Fisher's z-table.

Table I (5 per cent. probability) and Table II (1 per cent. probability) are for working with common logarithms (to the base "10"), and have been obtained directly from Fisher's Table VI (pp. 224—226) by multiplying "z" by $2 \log_{10}^{e} = 0{\cdot}86858896$*.

Illustration I.—In Fisher's Example 41 (Analysis of Variation in Experimental Field Trials, (1) pp. 205—209), the two observed variances (mean squares) are $v_1 = 3{\cdot}967$ and $v_2 = 0{\cdot}727$ based on $n_1 = 11$ and $n_2 = 24$ degrees of freedom. The common logarithms to the base 10 are

$$\log_{10} 3{\cdot}967 = 0{\cdot}59846$$
$$\log_{10} 0{\cdot}727 = \bar{1}{\cdot}86153$$
$$\text{Difference} = 0{\cdot}73693 = 2\,Z'$$

Looking up Table I, we notice that for $n_1 = 8$ and $n_2 = 24$, the 5 per cent. value of $2\,Z'$ is 0·3720. Further from Table II, for $n_1 = 8$ and $n_2 = 24$, the one per cent. point is 0·5267. The observed value of '$2\,Z'$' is 0·7369 for $n_1 = 11$ and $n_2 = 24$. The difference between the two observed variances is therefore definitely significant.

Illustration II.—In Fisher's Example 38 (Homogeneity of Small Samples, [1932] $v_1 = 60{\cdot}25$ ($n_1 = 24$), $v_2 = 31{\cdot}62$ ($n_2 = 3$). We find

$$\log_{10} 60{\cdot}25 = 1{\cdot}77996$$
$$\log_{10} 31.65 = 1.50037$$
$$\text{Difference} = 0{\cdot}27959 = 2\,Z'$$

In Table I, we find that for $n_1 = 24$ and $n_2 = 3$, $2\,Z' = 0{\cdot}9364$. The observed difference is, therefore, quite insignificant.

* The tabled values therefore give $2\,Z' = \log_{10}(s_1^2/s_2^2)$

The use of even common logarithm tables may be dispensed with if we use Table III (5 per cent. probability) and Table IV (1 per cent. probability) which give directly the value of the ratio of the variances, $x = (v_1/v_2) = (s_1^2/s_2^2)$

Illustration III.—In illustration I, we have $v_1 = 3{\cdot}967$ ($n_1 = 11$), and $v_2 = 0{\cdot}727$ ($n_2 = 24$). By direct division we find that the observed value of $v_1/v_2 = 5{\cdot}457$. In Table III (5 per cent. probability) we notice that for $n_1 = 8$, and $n_2 = 24$, expected $(v_1/v_2) = 2{\cdot}355$. Also in Table IV (1 per cent. probability) for $n_1 = 8$, $n_2 = 24$, $v_1/v_2 = 3{\cdot}363$. The observed difference is, therefore, definitely significant.

Illustration IV.—In the second illustration we had $v_1 = 60{\cdot}25$ ($n_1 = 24$), $v_2 = 31{\cdot}65$ ($n_2 = 3$). The observed ratio of v_1/v_2 is $1{\cdot}904$. In Table III (5 per cent. probability) the expected value of v_1/v_2 for $n_1 = 24$ and $n_2 = 3$ is $8{\cdot}638$. The observed difference is quite insignificant.

It will be noticed that in using Tables III and IV, it will usually be unnecessary to find v_1/v_2 with any great accuracy. For example in illustration III, we can see by mere inspection that the observed ratio of v_1/v_2 is greater than 5, and hence must be significant. So also in illustration IV, it is easy to see that v_1/v_2 is less than 2, and hence insignificant.

Finally, Table V and Table VI give the values of $y = S_1/S_2$ (*i.e.*, the ratio of the two standard deviations) at 5 per cent. and one per cent. levels of significance respectively.

Illustration V.—It is found [Mahalanobis, 1931] that the standard deviation for head-length for the Rabai tribe of East Africa is given by $S_1 = 7{\cdot}25$ cm. ($n_1 = 12$), and for the Nandi tribe is $S_2 = 4{\cdot}06$ cm. ($n_2 = 13$). The observed ratio of $(S_1/S_2) = 1{\cdot}786$.

In Table V (5 per cent. probability) for $n_1 = 12$ and $n_2 = 13$ the expected value of (S_1/S_2) is $1{\cdot}614$. But in Table VI (one per cent. probability) the expected value of (S_1/S_2) is $1{\cdot}9901$. We conclude that th probability of the difference being real lies between $0{\cdot}05$ and $0{\cdot}01$, and may be considered significant.

I need scarcely add that, just as in using Fisher's Tables, n_1 must refer to the larger of the two variances.

The Tables were computed by Babu Sudhir Kumar Banerjee and Babu Jitendra Mohan Sen Gupta in the Statistical Laboratory of the Presidency College with the aid of a grant from the Imperial Council of Agricultural Research.

TABLE I.

Five per cent. points of the distribution of $2z' = log_{10}(s_1^2/s_2^2)$.

Values of n_2	Values of n_1					
	1	2	3	4	5	
1	2·2080	2·2999	2·3339	2·3514	2·3620	
2	1·2674	1·2787	1·2825	1·2844	1·2855	
3	1·0056	·9801	·9673	·9599	·9549	
4	·8870	·8417	·8190	·8054	·7963	
5	·8200	·7624	·7332	·7154	·7033	
6	·7772	·7112	·6773	·6565	·6422	
7	·7475	·6755	·6382	·6150	·5990	
8	·7257	·6493	·6092	·5841	·5668	
9	·7090	·6290	·5869	·5602	·5418	
10	·6959	·6130	·5692	·5413	·5219	
11	·6852	·6001	·5548	·5259	·5057	
12	·6765	·5894	·5429	·5131	·4921	
13	·6691	·5804	·5328	·5023	·4808	
14	·6627	·5727	·5243	·4931	·4710	
15	·6573	·5661	·5168	·4851	·4626	
16	·6527	·5603	·5104	·4782	·4552	
17	·6485	·5553	·5047	·4720	·4487	
18	·6448	·5508	·4997	·4665	·4429	
19	·6415	·5468	·4952	·4617	·4378	
20	·6386	·5432	·4911	·4573	·4331	
21	·6360	·5399	·4875	·4533	·4289	
22	·6335	·5370	·4842	·4498	·4251	
23	·6314	·5343	·4812	·4465	·4216	
24	·6294	·5318	·4784	·4435	·4184	
25	·6276	·5296	·4758	·4407	·4154	
26	·6258	·5275	·4735	·4382	·4128	
27	·6243	·5256	·4714	·4358	·4102	
28	·6229	·5238	·4693	·4336	·4079	
29	·6215	·5221	·4675	·4316	·4057	
30	·6203	·5206	·4657	·4297	·4037	
60	·6022	·4984	·4406	·4023	·3744	
∞	·5845	·4765	·4158	·3751	·3452	

TABLE I—*contd.*

Five per cent. points of the distribution of $2z^1 = log_{10}(s_1^2/s_2^2)$—contd.

	Values of n_1					
	6	8	12	24	∞	
1	2·3692	2·3782	2·3872	2·3963	2·4054	
2	1·2862	1·2872	1·2881	1·2890	1·2899	
3	·9514	·9467	·9147	·9364	·9308	
4	·7898	·7811	·7717	·7615	·7504	
5	·6946	·6829	·6700	·6558	·6400	
6	·6318	·6177	·6020	·5845	·5645	
7	·5873	·5712	·5532	·5328	·5092	
8	·5540	·5364	·5163	·4935	·4665	
9	·5281	·5092	·4875	·4624	·4325	
10	·5075	·4874	·4643	·4373	·4045	
11	·4906	·4696	·4452	·4165	·3810	
12	·4766	·4546	·4292	·3989	·3610	
13	·4647	·4420	·4156	·3838	·3437	
14	·4545	·4312	·4038	·3708	·3285	
15	·4457	·4217	·3936	·3594	·3151	
16	·4379	·4134	·3846	·3493	·3031	
17	·4312	·4062	·3767	·3404	·2924	
18	·4251	·3997	·3696	·3324	·2826	
19	·4197	·3939	·3632	·3251	·2737	
20	·4148	·3886	·3575	·3186	·2655	
21	·4104	·3839	·3522	·3126	·2581	
22	·4064	·3796	·3475	·3071	·2512	
23	·4027	·3757	·3431	·3021	·2448	
24	·3994	·3720	·3391	·2975	·2388	
25	·3963	·3686	·3354	·2932	·2332	
26	·3934	·3656	·3321	·2892	·2280	
27	·3904	·3627	·3288	·2855	·2231	
28	·3883	·3601	·3259	·2821	·2185	
29	·3860	·3576	·3231	·2789	·2142	
30	·3839	·3552	·3206	·2759	·2101	
60	·3530	·3216	·2827	·2305	·1428	
∞	·3290	·2874	·2437	·1811	..	

Values of n_2

TABLE II.

One per cent. points of distribution of $2z^1 = log_{10}(s_1^2/s_2^2)$.

Values of n_2	Values of n_1				
	1	2	3	4	5
1	3·6077	3·6989	3·7327	3·7501	3·7607
2	1·9934	1·9957	1·9964	1·9967	1·9970
3	1·5330	1·4888	1·4692	1·4580	1·4508
4	1·3263	1·2553	1·2225	1·2035	1·1909
5	1·2111	1·1230	1·0813	1·0566	1·0400
6	1·1381	1·0384	·9903	·9614	·9418
7	1·0880	·9798	·9270	·8946	·8728
8	1·0515	·9369	·8803	·8455	·8216
9	1·0237	·9043	·8446	·8077	·7823
10	1·0019	·8785	·8164	·7777	·7510
11	·9844	·8576	·7935	·7534	·7256
12	·9699	·8405	·7747	·7334	·7045
13	·9578	·8261	·7589	·7164	·6868
14	·9475	·8139	·7453	·7020	·6716
15	·9387	·8034	·7338	·6896	·6586
16	9310	·7942	·7236	·6787	·6471
17	·9243	·7862	·7148	·6692	·6371
18	·9183	·7791	·7068	·6607	·6282
19	·9130	·7728	·6998	·6533	·6202
20	·9083	·7671	·6936	·6465	·6130
21	·9040	·7619	·6879	·6403	·6066
22	·9001	·7573	·6827	·6348	·6007
23	·8966	·7531	·6780	·6298	·5954
24	·8933	·7492	·6738	·6251	·5906
25	·8904	·7457	·6698	·6209	·5860
26	·8877	·7425	·6662	6170	·5819
27	·8852	·7394	·6628	·6134	·5780
28	·8828	·7366	·6597	·6100	·5745
29	·8807	·7340	·6568	·6069	·5712
30	·8787	·7316	·6541	·6040	·5681
60	·8498	·6970	·6155	·5622	·5236
∞	·8218	·6633	·5777	·5211	·4796

TABLE II—*contd.*

One per cent. points of distribution of $2z^1 = \log_{10}(s_1^2/s_2^2)$—contd.

	Values of n_1					
	6	8	12	24	∞	
1	3·7679	3·7768	3·7857	3·7948	3·8039	
2	1·9971	1·9972	1·9975	1·9977	1·9978	
3	1·4458	1·4392	1·4322	1·4248	1·4170	
4	1·1821	1·1703	1·1576	1·1439	1·1292	
5	1·0282	1·0114	·9951	·9762	·9552	
6	·9276	·9085	·8875	·8641	·8376	
7	·8568	·8351	·8108	·7835	·7520	
8	·8042	·7803	·7533	·7226	·6865	
9	·7636	·7378	·7085	·6748	·6345	
10	·7313	·7039	·6726	·6362	·5920	
11	·7049	·6762	·6432	·6044	·5566	
12	·6831	·6532	·6186	·5775	·5264	
13	·6646	·6336	·5978	5·547	·5004	Values of n_2
14	·6489	·6170	·5798	·5350	·4777	
15	·6353	·6025	·5642	·5178	·4577	
16	·6234	·5898	·5506	·5026	·4398	
17	·6130	·5787	·5384	·4890	·4238	
18	·6037	·5688	·5277	·4791	·4093	
19	·5953	·5600	·5180	·4661	·3961	
20	·5879	·5520	·5093	·4563	·3840	
21	·5811	·5448	·5014	·4473	·3730	
22	·5750	·5382	·4943	·4392	·3627	
23	·5694	·5322	·4877	·4316	·3533	
24	·5642	·5267	·4816	·4247	·3446	
25	·5595	·5217	·4761	·4183	·3363	
26	·5552	·5170	·4709	·4124	·3287	
27	·5512	·5126	·4662	·4069	·3215	
28	·5475	·5086	·4617	·4018	·3148	
29	·5440	·5049	·4577	·3969	·3083	
30	·5408	·5014	·4538	·3925	·3024	
60	·4940	·4507	.3973	·3254	·2043	
∞	·4475	·3999	·3394	·2530	0	

TABLE III.

Five per cent. points of the distribution of the ratio of variance, $x = (s_1^2/s_2^2)$.

	Values of n_1					
	1	2	3	4	5	
1	161·45	199·50	215·72	224·57	230·17	
2	18·512	18·999	19·163	19·248	19·298	
3	10·129	9·552	9·276	9·118	9·014	
4	7·710	6·945	6·591	6·388	6·257	
5	6·607	5·786	5·410	5·192	5·050	
6	5·987	5·143	4·756	4·534	4·388	
7	5·591	4·732	4·347	4·121	3·972	
8	5·317	4·459	4·067	3·838	3·688	
9	5·117	4·256	3·863	3·633	3·482	
10	4·965	4·103	3·708	3·478	3·326	
11	4·844	3·982	3·587	3·357	3·204	
12	4·747	3·885	3·490	3·259	3·106	
13	4·667	3·805	3·410	3·179	3·025	
14	4·600	3·739	3·344	3·112	2·958	
15	4·543	3·683	3·287	3·056	2·901	Values of n_2
16	4·494	3·634	3·239	3·007	2·853	
17	4·451	3·592	3·197	2·965	2·810	
18	4·414	3·555	3·160	2·928	2·773	
19	4·381	3·522	3·127	2·895	2·740	
20	4·351	3·493	3·098	2·866	2·711	
21	4·325	3·467	3·072	2·840	2·685	
22	4·301	3·443	3·049	2·817	2·661	
23	4·279	3·422	3·028	2·795	2·640	
24	4·260	3·403	3·009	2·777	2·621	
25	4·242	3·385	2·991	2·759	2·603	
26	4·225	3·369	2·975	2·743	2·587	
27	4·210	3·354	2·961	2·738	2·572	
28	4·196	3·340	2·947	2·714	2·558	
29	4·183	3·328	2·934	2·702	2·545	
30	4·171	3·316	2·922	2·690	2·534	
60	4·001	3·151	2·758	2·525	2·368	
∞	3·841	2·996	2·605	2·372	2·214	

TABLE III—*contd.*

Five per cent. points of the distribution of the ratio of variance, $x = (s_1^2/s_2^2)$—contd.

	Values of n_1					
	6	8	12	24	∞	
1	233·97	238·89	243·91	249·04	254·32	
2	19·329	19·371	19·414	19·453	19·496	
3	8·941	8·844	8·744	8·638	8·527	
4	6·164	6·041	5·912	5·774	5·628	
5	4·950	4·818	4·678	4·527	4·365	
6	4·284	4·144	4·000	3·841	3·669	
7	3·866	3·725	3·574	3·410	3·230	
8	3·580	3·438	3·284	3·116	2·928	
9	3·374	3·230	3·073	2·900	2·707	
10	3·217	3·072	2·913	2·737	2·538	
11	3·094	2·948	2·788	2·609	2·405	
12	2·999	2·848	2·686	2·506	2·296	
13	2·915	2·767	2·604	2·420	2·207	Values of n_2
14	2·848	2·699	2·534	2·349	2·131	
15	2·790	2·641	2·475	2·288	2·066	
16	2·741	2·591	2·424	2·235	2·010	
17	2·699	2·548	2·381	2·190	1·961	
18	2·661	2·510	2·342	2·150	1·917	
19	2·629	2·477	2·308	2·114	1·878	
20	2·599	2·447	2·278	2·083	1·843	
21	2·573	2·421	2·250	2·054	1·812	
22	2·549	2·397	2·226	2·028	1·783	
23	2·528	2·375	2·203	2·005	1·757	
24	2·508	2·355	2·183	1·984	1·733	
25	2·490	2·337	2·165	1·965	1·711	
26	2·474	2·321	2·148	1·947	1·691	
27	2·459	2·305	2·132	1·930	1·672	
28	2·445	2·292	2·118	1·915	1·654	
29	2·432	2·278	2·104	1·901	1·638	
30	2·421	2·266	2·092	1·887	1·622	
60	2·254	2·097	1·918	1·700	1·389	
∞	2·099	1·938	1·752	1·517	1·000	

TABLE IV.

One per cent. points of the distribution of the ratio of variance, $x = (s_1^2/s_2^2)$.

Values of n_2	Values of n_1					
	1	2	3	4	5	
1	4052·1	4999·0	5403·5	5625·1	5764·1	
2	98·495	99·008	99·167	99·237	99·305	
3	34·117	30·815	29·459	28·709	28·236	
4	21·200	18·001	16·693	15·978	15·521	
5	16·258	13·274	12·059	11·391	10·966	
6	13·744	10·924	9·779	9·149	8·746	
7	12·246	9·546	8·452	7·846	7·460	
8	11·259	8·649	7·591	7·006	6·631	
9	10·561	8·022	6·992	6·423	6·057	
10	10·044	7·560	6·552	5·994	5·636	
11	9·647	7·205	6·217	5·668	5·317	
12	9·330	6·927	5·953	5·412	5·064	
13	9·074	6·701	5·740	5·205	4·862	
14	8·862	6·514	5·563	5·035	4·695	
15	8·683	6·359	5·417	4·893	4·556	
16	8·532	6·227	5·292	4·772	4·437	
17	8·400	6·112	5·185	4·669	4·336	
18	8·285	6·013	5·092	4·579	4·248	
19	8·184	5·926	5·010	4·501	4·170	
20	8·096	5·849	4·938	4·431	4·103	
21	8·017	5·780	4·875	4·368	4·042	
22	7·944	5·719	4·816	4·314	3·988	
23	7·881	5·663	4·765	4·264	3·939	
24	7·823	5·614	4·718	4·218	3·895	
25	7·770	5·568	4·676	4·177	3·855	
26	7·722	5·527	4·637	4·140	3·818	
27	7·677	5·488	4·601	4·106	3·785	
28	7·636	5·453	4·568	4·074	3·754	
29	7·597	5·421	4·538	4·045	3·726	
30	7·563	5·390	4·510	4·018	3·699	
60	7·077	4·978	4·126	3·649	3·339	
∞	6·635	4·605	3·782	3·320	3·017	

TABLE IV—*contd.*

One per cent. points of the distribution of the ratio of variance, $x = (s_1^2/s_2^2)$—contd.

Values of n_2	Values of n_1					
	6	8	12	24	∞	
1	5859·4	5981·4	6105·8	6234·2	6366·5	
2	99·325	99·365	99·425	99·464	99·504	
3	27·910	27·489	27·053	26·597	26·122	
4	15·208	14·800	14·374	13·930	13·271	
5	10·672	10·266	9·888	9·467	9·019	
6	8·465	8·101	7·718	7·313	6·880	
7	7·191	6·840	6·458	6·044	5·650	
8	6·371	6·029	5·641	5·279	4·859	
9	5·802	5·467	5·111	4·730	4·311	
10	5·386	5·057	4·706	4·327	3·909	
11	5·069	4·745	4·397	4·021	3·520	
12	4·820	4·500	4·156	3·780	3·361	
13	4·620	4·302	3·961	3·586	3·165	
14	4·456	4·140	3·800	3·427	3·005	
15	4·318	4·004	3·668	3·294	2·869	
16	4·201	3·889	3·553	3·181	2·753	
17	4·102	3·791	3·455	3·083	2·653	
18	4·015	3·706	3·370	3·014	2·566	
19	3·939	3·631	3·296	2·925	2·489	
20	3·871	3·565	3·231	2·859	2·421	
21	3·811	3·506	3·173	2·801	2·360	
22	3·759	3·453	3·121	2·749	2·305	
23	3·710	3·406	3·074	2·702	2·256	
24	3·666	3·363	3·031	2·659	2·210	
25	3·627	3·324	2·993	2·620	2·169	
26	3·591	3·288	2·958	2·585	2·132	
27	3·558	3·256	2·925	2·551	2·096	
28	3·528	3·226	2·896	2·522	2·064	
29	3·499	3·198	2·869	2·494	2·034	
30	3·474	3·173	2·843	2·469	2·006	
60	3·119	2·823	2·496	2·115	1·601	
∞	2·802	2·511	2·182	1·791	1·000	

TABLE V.

Five per cent. points of the distribution of the ratio of standard deviations, $y = (s_1/s_2)$

Values of n_2	Values of n_1					
	1	2	3	4	5	
1	12·706	14·124	14·688	14·986	15·171	
2	4·3025	4·3591	4·3776	4·3873	4·3929	
3	3·1826	3·0907	3·0456	3·0195	3·0024	
4	2·7766	2·6353	2·5674	2·5274	2·5013	
5	2·5764	2·4054	2·3258	2·2787	2·2472	
6	2·4468	2·2678	2·1810	2·1293	2·0946	
7	2·3646	2·1765	2·0839	2·0293	1·9929	
8	2·3060	2·1117	2·0166	1·9591	1·9203	
9	2·2621	2·0631	1·9654	1·9060	1·8660	
10	2·2282	2·0255	1·9257	1·8649	1·8238	
11	2·2009	1·9955	1·8940	1·8322	1·7900	
12	2·1788	1·9711	1·8682	1·8053	1·7623	
13	2·1604	1·9507	1·8466	1·7830	1·7393	
14	2·1447	1·9336	1·8287	1·7642	1·7200	
15	2·1314	1·9190	1·8130	1·7480	1·7034	
16	2·1200	1·9062	1·7997	1·7341	1·6889	
17	2·1098	1·8951	1·7880	1·7218	1·6763	
18	2·1009	1·8853	1·7777	1·7110	1·6651	
19	2·0930	1·8767	1·7684	1·7015	1·6553	
20	2·0859	1·8690	1·7602	1·6930	1·6464	
21	2·0796	1·8619	1·7528	1·6852	1·6385	
22	2·0738	1·8556	1·7461	1·6783	1·6313	
23	2·0687	1·8498	1·7402	1·6720	1·6248	
24	2·0649	1·8447	1·7346	1·6663	1·6188	
25	2·0596	1·8399	1·7294	1·6610	1·6133	
26	2·0555	1·8355	1·7248	1·6562	1·6083	
27	2·0518	1·8314	1·7206	1·6515	1·6037	
28	2·0485	1·8276	1·7165	1·6474	1·5994	
29	2·0452	1·8241	1·7129	1·6436	1·5954	
30	2·0423	1·8210	1·7095	1·6400	1·5917	
60	2·0003	1·7750	1·6608	1·5891	1·5389	
∞	1·9599	1·7308	1·6140	1·5402	1·4879	

TABLE V—*contd.*

Five per cent. points of the distribution of the ratio of standard deviations, $y=(s_1/s_2)$—contd.

	Values of n_1					
	6	8	12	24	∞	
1	15·296	15·456	15·618	15·781	15·948	
2	4·3965	4·4013	4·4061	4·4106	4·4154	
3	2·9901	2·9740	2·9571	2·9391	2·9200	
4	2·4826	2·4579	2·4315	2·4030	2·3724	
5	2·2249	2·1950	2·1628	2·1276	2·0892	
6	2·0697	2·0364	1·9999	1·9599	1·9153	
7	1·9662	1·9302	1·8906	1·8467	1·7971	
8	1·8923	1·8543	1·8121	1·7651	1·7110	
9	1·8368	1·7971	1·7529	1·7030	1·6453	
10	1·7937	1·7526	1·7068	1·6545	1·5931	
11	1·7591	1·7170	1·6696	1·6153	1·5507	
12	1·7310	1·6878	1·6390	1·5828	1·5153	
13	1·7074	1·6635	1·6137	1·5557	1·4854	
14	1·6876	1·6428	1·5919	1·5325	1·4597	
15	1·6705	1·6250	1·5733	1·5126	1·4373	
16	1·6557	1·6096	1·5571	1·4951	1·4177	
17	1·6428	1·5962	1·5430	1·4798	1·4002	
18	1·6313	1·5844	1·5304	1·4662	1·3844	
19	1·6213	1·5738	1·5192	1·4540	1·3704	
20	1·6122	1·5642	1·5092	1·4431	1·3576	
21	1·6040	1·5558	1·5001	1·4332	1·3460	
22	1·5966	1·5481	1·4920	1·4242	1·3354	
23	1·5898	1·5411	1·4844	1·4160	1·3255	
24	1·5838	1·5346	1·4776	1·4085	1·3164	
25	1·5781	1·5287	1·4714	1·4016	1·3080	
26	1·5729	1·5233	1·4657	1·3951	1·3002	
27	1·5682	1·5183	1·4602	1·3892	1·2929	
28	1·5638	1·5138	1·4553	1·3838	1·2861	
29	1·5596	1·5094	1·4505	1·3786	1·2797	
30	1·5558	1·5053	1·4464	1·3738	1·2737	
60	1·5014	1·4480	1·3847	1·3040	1·1787	
∞	1·4486	1·3922	1·3237	1·2319	1·000	

Values of n_2

TABLE VI.

One per cent. points of the distribution of the ratio of standard deviations, $y = (s_1/s_2)$.

Values of n_2	Values of n_1					
	1	2	3	4	5	
1	63·657	70·704	73·508	75·001	75·922	
2	9·9244	9·9503	9·9582	9·9617	9·9652	
3	5·7741	5·5511	5·4276	5·3581	5·3138	
4	4·6043	4·2427	4·0857	3·9972	3·9397	
5	4·0322	3·6433	3·4726	3·3750	3·3115	
6	3·7073	3·3052	3·1271	3·0247	2·9573	
7	3·4994	3·0898	2·9072	2·8011	2·7314	
8	3·3555	2·9408	2·7552	2·6469	2·5751	
9	3·2498	2·8323	2·6443	2·5343	2·4611	
10	3·1693	2·7494	2·5597	2·4483	2·3741	
11	3·1464	2·6842	2·4933	2·3807	2·3057	
12	3·0544	2·6319	2·4398	2·3264	2·2504	
13	3·0123	2·5886	2·3958	2·2814	2·2049	
14	2·9770	2·5523	2·3587	2·2439	2·1667	
15	2·9467	2·5216	2·3275	2·2120	2·1344	
16	2·9209	2·4953	2·3004	2·1845	2·1064	
17	2·8982	2·4722	2·2876	2·1608	2·0824	
18	2·8783	2·4522	2·2565	2·1398	2·0610	
19	2·8608	2·4344	2·2383	2·1215	2·0421	
20	2·8454	2·4184	2·2222	2·1050	2·0255	
21	2·8315	2·4042	2·2078	2·0901	2·0105	
22	2·8188	2·3914	2·1946	2·0770	1·9969	
23	2·8072	2·3798	2·1828	2·0649	1·9848	
24	2·7969	2·3693	2·1721	2·0538	1·9737	
25	2·7874	2·3596	2·1624	2·0438	1·9634	
26	2·7787	2·3509	2·1533	2·0346	1·9540	
27	2·7707	2·3427	2·1449	2·0263	1·9455	
28	2·7632	2·3352	2·1372	2·0184	1·9375	
29	2·7563	2·3282	2·1302	2·0111	1·9302	
30	2·7500	2·3217	2·1236	2·0045	1·9232	
60	2·6602	2·2311	2·0311	1·9102	1·8272	
∞	2·5759	2·1460	1·9447	1·8219	1·7371	

TABLE VI—*contd.*

One per cent. points of the distribution of the ratio of standard deviations. $y=(s_1/s_2)$—contd.

n_2	Values of n_1					
	6	8	12	24	∞	
1	76·547	77·339	78·140	78·957	79·790	
2	9·9662	9·9682	9·9712	9·9732	9·9752	
3	5·2830	5·2430	5·2013	5·1572	5·1110	
4	3·8997	3·8470	3·7913	3·7322	3·6693	
5	3·2668	3·2040	3·1446	3·0768	3·0033	
6	2·9096	2·8462	2·7782	2·7042	2·6230	
7	2·6816	2·6154	2·5434	2·4645	2·3769	
8	2·5241	2·4554	2·3805	2·2977	2·2043	
9	2·4087	2·3382	2·2608	2·1747	2·0761	
10	2·3208	2·2488	2·1693	2·0801	1·9770	
11	2·2515	2·1782	2·0970	2·0053	1·8980	
12	2·1955	2·1212	2·0385	1·9443	1·8333	
13	2·1494	2·0740	1·9901	1·8879	1·7791	
14	2·1109	2·0346	1·9494	1·8513	1·7333	
15	2·0780	2·0011	1·9148	1·8150	1·6937	
16	2·0497	1·9721	1·8849	1·7835	1·6593	
17	2·0253	1·9470	1·8587	1·7559	1·6289	
18	2·0037	1·9249	1·8358	1·7360	1·6019	
19	1·9846	1·9054	1·8159	1·7102	1·5777	
20	1·9676	1·8879	1·7975	1·6910	1·5560	
21	1·9523	1·8724	1·7812	1·6736	1·5363	
22	1·9387	1·8582	1·7667	1·6580	1·5183	
23	1·9261	1·8454	1·7533	1·6436	1·5029	
24	1·9148	1·8338	1·7411	1·6307	1·4869	
25	1·9045	1·8232	1·7300	1·6187	1·4729	
26	1·8950	1·8134	1·7198	1·6077	1·4599	
27	1·8863	1·8043	1·7104	1·5976	1·4479	
28	1·8782	1·7961	1·7017	1·5882	1·4368	
29	1·8707	1·7884	1·6937	1·5793	1·4262	
30	1·8638	1·7812	1·6861	1·5713	1·4164	
60	1·7660	1·6802	1·5800	1·4544	1·2652	
∞	1·6740	1·5847	1·4782	1·3382	1·1000	

Values of n_2

REFERENCES.

Fisher, R. A. (1932). Statistical Methods for Research Workers, 4th edition, p. 208.

Mahalanobis, P. C. (1931). *Rec. Ind. Mus.* **33**, 145.

STATISTICAL NOTES FOR AGRICULTURAL WORKERS.

NO. 4.—RICE AND POTATO EXPERIMENTS AT SRINIKETAN (AGRICULTURAL DEPARTMENT OF THE VISVABHARATI), 1931.

BY

P. C. MAHALANOBIS.

(Received for publication on the 18th August 1932)

INTRODUCTION.

The Sriniketan Farm is situated in the Birbhum district of Bengal, at a distance of about $1\frac{1}{2}$ mile from Bolpur station of the E. I. Railway. Paddy and potato form two of the staple crops of this locality. On account of their economic importance these two crops were selected by the authorities of the Department of Rural Reconstruction of the Visvabharati for a preliminary experiment in 1931. Owing to limited financial resources it was decided to restrict the size of the fields to two *bighas* and one *bigha* ($\frac{2}{3}$ and $\frac{1}{3}$ acre) for paddy and potato respectively.

The experimental fields were laid out on virgin soil and the experiments were conducted by Messrs. Hashim Ali, Santosh Behari Bose, and Visvanath Chatterjee under the general direction of Mr. Gour Gopal Ghosh.

Fisher's method of Randomized Blocks was used in designing the lay-out which was prepared in the Statistical Laboratory of the Presidency College. The statistical analyis was carried out by Mr. Subhendu Sekhar Bose and other workers of the same laboratory.

PADDY.

The Sriniketan Farm lies in a raised undulating country with a sandy soil and a sub-soil of red laterite. The soil is very poor in organic matter, and has little retentive power. Climatic conditions are comparatively dry for Bengal, the normal rainfall in this region being about 50 in. per year of which 39 in. fall during the monsoon months of June, July, August, and September. The monthly distribution is given below in Table R. The soil is of the kind usually called "high poor" land for paddy.

TABLE R.

Normal rainfall in Bolpur in inches.

	in.		in.
January	0·41	July	11·14
February	0·72	August	10·74
March	0·98	September	8·00
April	1·06	October	3·32
May	3·84	November	0·55
June	9·43	December	0·05

Varieties.—Much of the land in the Birbhum district belongs to this "high poor" type. But large tracts of "medium" and "low" lands also occur in the same district. In fact, such lands exist in the immediate neighbourhood of the Sriniketan Farm. For the preliminary experiment it was, therefore, decided to try 3 varieties specially suited for these three types of land.

Red Aus is an early variety mostly grown on "high poor" land. On account of its early character it is largely used for actual consumption in the villages when the supply of paddy runs low. It also leaves sufficient time (and moisture in the soil) to enable a *rabi* crop being grown on the same field.

Kashiphul is grown on "medium" land, and is a comparatively late variety. The yield is higher, and the quality of the grain much better than Red Aus. It is usually grown on much better land, and a second crop of potato or onion is usually taken from the same field.

Dudkalma is a late, high-yielding variety grown on "low" land. The grains are fine and fetch higher prices than either Red Aus or Kashiphul, and are usually exported from the villages for outside sale.

Manures.—Owing to the virgin character of the soil cow-dung was applied heavily to all the plots at the rate of 10 baskets (1 basket =20 seers) per plot of 39 ft. × 12 ft. or 15·5 mds. per *bigha* or 46·4 mds. (166·6 tons) per acre.

Two fertilizers were tried. Ammophos was applied at the rate of 4 *ch.* per plot or 6 seers and 12 chittacks per *bigha* or 40·5 lbs. per acre at the time of pudding on the 25th and 26th July, 1931.

Sulphate of ammonia was given as a top dressing at the rate of 3 *ch.* per plot, *i.e.* 5 seers 1 *ch.* per *bigha* or 15 seers 3 *ch.* per acre at the time of hoeing on the 1st and 2nd September.

The size of the field for the paddy experiment was about 2 *bighas* or $\frac{2}{3}$ acre. It was divided into 6 blocks each consisting of 9 plots of size 39 ft. × 12 ft. ($\frac{1}{3^1}$ *bigha* or $\frac{1}{9^3}$ acre each approximately). The 3 varieties with 3 manures (including 1

control) were distributed at random within each block, so that each treatment with each variety was replicated 6 times. Details are shown in Table I.

TABLE I.

Paddy experiment in Bolpur 1931-32. Yield of grain in chattaks *per plot of* $\frac{1}{3^{rd}}$ bigha.

Treatment		I	II	III	IV	V	VI	Total
Red Aus	Ammophos.	112	128	118	128	92	152	730
	Amon. sulph.	168	116	144	100	100	80	708
	Control	106	84	68	156	156	128	698
Kashiphul	Ammophos.	112	81	108	96	53	48	498
	Amon. sulph.	61	98	58	86	65	98	466
	Control	97	86	92	80	99	66	520
Dudkalma	Ammophos.	134	112	116	114	101	128	705
	Amon. sulph.	125	106	110	102	56	110	609
	Control	62	60	99	90	58	87	456
	Total	977	871	913	952	780	897	5,390

Progress of the experiment.—The field was prepared and laid out in June just before the break of the monsoon. As no experiment with fertilizers had been carried out in this field so far, there was no question of any residual effects from previous season.

The dates of various operations are given below.

TABLE II.

Dates of operations.

Operation	Red Aus	Kashiphul	Dudkalma
1. Sowing	15th June	15th June	29th June
2. Transplanting	25th July	and 26th July	(all varieties)
3. Ammophos	,,	,, ,,	,,
4. Amon. sulphate	1st September	,, 2nd September	,,
5. Weeding and hoeing	1st, 2nd and 3rd September		,,
6. Flowering	26th September	24th September	3rd November
7. Harvesting	31st October	16th November	7th and 8th December

The yield of grain in each plot is shown in figures in Table I.
The analysis of variance is given in Table III.

TABLE III.

Analysis of variance.

—	Degrees of freedom	Sum of squares	Mean square	Standard deviation	½ $\log_e$ (mean square)
Varieties . . .	2	11867	5933·50	77·03	4·3442
Manures . . .	2	1879	939·50	30·65	3·4227
Differential response of varieties to manures	4	3806	951·50	30·85	3·4290
Treatment . . .	8	17552	2194·00	46·84	...
Blocks	5	2670	534·00	23·11	...
Errors	40	22574	564·35	23·76	3·1678
Total .	53	42796	807·47	28·42	

We find that the mean variance for fluctuations between "blocks" is 534·00 against a residual variance of 564·35. It is clear, therefore, that the division into "blocks" has not helped in any way in eliminating the effect of soil heterogeneity. It is possible that the variations in fertility in different portions of the field are not systematic.

We may now proceed with Fisher's z-test of significance for comparison of the different variances.

Let S_1 and S_2 be the two variances under comparison. Then "Z" is defined to be $\frac{1}{2}$ ($\log_e S_1^2$—$\log_e S_2^2$) where the logarithms are natural, *i.e.*, calculated to the base "e". These values are given in the last column of Table III.

For example for "varietal" differences we have $\log_e 5933{\cdot}5 = 8{\cdot}6884$ and for residual errors $\log_e 564{\cdot}35 = 6{\cdot}3356$; so that the difference of the natural logarithms is 2·3528, and 'Z' is given by half the difference or 1·1764. It will be noticed that the two variances are based on 2 and 40 degress of freedom respectively.

In the same way we obtain the value of 'Z' (and the corresponding degrees of freedom) for "manurial" differences, as well as for the "differential action of manures."

TABLE IV.

Values of 'Z'.

	n_1	n_2	Observed	Expected 5 per cent.
Variety	2	40	1·1764	0·5866
Manure	2	40	0·2549	0·5866
Differential response . .	4	40	0·2612	0·4789

The observed values of 'z' are shown in Table IV. We can easily find the corresponding critical values from Fisher's Table VI (Statistical Methods for Research Workers, 1932, p. 224-27). For example, for n_1=2 and n_2=40, we notice that the other critical values of 'z' are obtained in the same way, and shown in column 5 of Table IV.

For varietal trials the 5 per cent. value of 'z'=·5866. This implies, that, in case the varietal differences are really *nil*, the observed value of 'z' will exceed ·5866 only once in twenty trials. But the observed value is 1·1764. That is, the odds are more than 20 to 1 in favour of the varietal differences being real.

It will be noticed, however, that the "manurial" or "differential" effects cannot be considered significant. Full details of the different effects are given in Tables V-VII.

TABLE V.

Mean yield of different varieties of paddy.

	Chattaks per plot	Maunds per *bighà*	lbs. per acre.	Per cent.
Red Aus	118·7	5·75	1379·89	118·8
Kashiphul	82·4	3·99	957·90	83·5
Dudkalma	98·3	4·76	1142·74	98·4
Mean	99·8	4·83	1160·18	100·0
S. E.	5·6	0·27	65·10	6·0

It is clear from Table V that in the "high poor" land of Sriniketan Farm the yield of Red Aus comes out best, while Kashiphul is distinctly the worst. The difference between Red Aus and Kashiphul is 35·3 per cent., between Red Aus and Dudkalma 20·4 per cent., and between Dudkalma and Kashiphul 15·9 per cent., while the effective precision of the experiment is given by a standard error of 6·0 per cent. for a single mean based on 18 replications.

TABLE VI.

Mean yield of paddy for different manurial treatments.

	Chattak per $\frac{1}{3\cdot1}$ *bigha*	Yield in mds. per *bigha*	Lbs. per acre	Per cent.
Ammophos	107·4	5·20	1248·5	108·0
Ammonium sulphate . .	99·1	4·80	1152·0	99·0
Control (No manure) . .	93·0	4·50	1081·1	93·0
Mean	99·8	4·83	1160·2	100·0
S. E.	5·6	0·27	65·1	6·0

The effect of manures is not generally significant. Table VI shows, however, that the difference between ammophos and the control (no fertilizer) is quite large being 14·4 per cent. with a standard error of 6·0 per cent. This difference is suggestive, but the effect of sulphate of ammonia is inappreciable.

TABLE VII.

Mean yield in chattaks per plot of $\frac{1}{3\cdot1}$ bigha.

	Ammophos	Ammonium sulphate	Control	Mean
Red *aus*	121·6	118·0	116·3	118·7
Kashiphul	83·0	77·8	86·7	82·4
Dudkalma	117·5	101·5	76·0	98·3
Mean	107·4	99·1	93·0	99·8

Standard error of mean of 6 plots = 9·7.

Finally Table VII shows the full analysis for each variety and each manure. The effect of fertilizers is entirely negligible in the case of both red *aus* and *kashiphul*. *Dudkalma*, however, appears to show a definite response to sulphate of ammonia, and an increased response to ammophos. This result is interesting; *dudkalma* which requires a richer soil, appears to be able to derive a certain amount of additional nourishment from the chemical fertilizers. This point deserves further attention.

The results of the experiment may now be summarized. In the "high poor" land of the Sriniketan Farm, Red Aus appears to do best and Kashiphul worst, Dudkalma coming between the two. The effect of chemical fertilizers is not quite clear, being generally inappreciable. Dudkalma, however, appears to show better results with sulphate of ammonia, and still more so with ammophos.

On the technical side the division into " blocks " did not lead to added precision owing probably to the variation in soil fertility being unsystematic. The residual standard deviation is 23·76 chattaks per plot for a mean yield of 99·81 chattaks per plot or a standard deviation of about 23·8 per cent. This is very high and may be compared with a residual standard deviation only 6·7 per cent. in the Mandalay Paddy Experiments [1932]. Evidently the fertility of the Sriniketan field varies very considerably from plot to plot.

The effective precision of comparision is given by a standard error of 5·6 per cent. for a single mean based on 18 replications which does not compare very unfavourably with a standard error of mean of 3·1 per cent. in the C. P. Rice Experiments [1931] and 2·72 per cent. in the Mandalay Rice Experiments.

Potato.

Like the rice experiment 3 varieties were chosen for the potato experiment. " Deshi " is an early variety largely grown in the locality and has good keeping qualities. " Patna " is a heavy yielding type with a bigger size of potatoes. " Darjeeling " is a special variety much favoured for both yield and quality.

The size of the field for the potato experiment was about 1 *bigha* ($\frac{1}{3}$ acre). It was divided into three blocks with 9 plots each of size 39 ft. × 12 ft. ($\frac{1}{3\,1}$ *bigha* or $\frac{1}{9\,3}$ acre). The lay-out is shown in Table VIII. This field also like the rice field was virgin soil.

Table VIII.

Lay-out and yield of potato experiment. Area of plot =39 ft.×12 ft.=$\frac{1}{3\,1}$ of a bigha *or $\frac{1}{9\,3}$ of an acre (Yield in seers and chattaks).*

BX	AO	CY	CX	AO	BY	AX	CY	BO
6—0	3—13	5—8	5—4	3—4	5—8	5—2	7—8	6—0
AY	CX	BO	BO	CY	AX	BY	AO	CX
4—4	6—6	6—8	5—2	4—2	2—10	4—8	2—8	10—4
CO	BY	AX	AY	BX	CO	CO	BX	AY
6—2	5—12	1—3	2—4	4—12	3—14	5—4	4—8	2—2

A—Darjeeling
B—Patna
C—Deshi

O=No fertilizer
Y=Potassium nitrate
X=Ammonium phosphate

All the plots were treated with cow-dung at the rate of 10 baskets or 20 seers per plot or 15·5 mds. per *bigha*, *i.e.*, 16·6 tons per acre. The artificial fertilizers used were ammophos at the rate of 1 seer 9 chattaks per plot, *i.e.*, 42 seers 3 chattaks per *bigha* or 3 mds. 6 seers and 9 chattaks per acre and potassium nitrate at the rate of 1 seer 14 chattaks per plot or 1 md. 10 seers and 10 chattaks per *bigha*, or 3 mds. 31 seers and 14 chattaks per acre.

The seeds were sown on the 14th and 15th November, 1931, at a distance of 9 in. in rows placed 1 ft. 9 in. from one another. The chemical fertilizers were applied and earthed on the 25th and 26th December. "Deshi" and "Patna" varieties were harvested from the 3rd to the 5th February 1932, and the "Darjeeling" variety as late as the 23rd March owing to the late development of the tubers. The actual yield in seers and chattaks are given for each plot in Table VIII.

One most important to be noted in this experiment was the complete absence of artificial irrigation. Rainfall was also scanty, the actual amount being *nil* in December and January.

The analysis of variance is given in Table IX.

TABLE IX.

Analysis of variance (*Potato*).

	Degrees of freedom	Sum of squares	Mean square	S. D.	$\log_e$ (mean square)
Varieties	2	10095	5047·50	71·04	8·5265
Manures	2	614	307·00	17·52	5·7266
Differential response of varieties to manure	4	3443	860·75	29·34	6·7581
Blocks	2	1374	687·00	26·21	...
Errors	16	9123	570·20	23·88	6·3460
Total .	26	24649	948·04	30·79	..

The "*Z*" values are given in Table X.

TABLE X.

Values of "Z".

—	n_1	n_2	Observed	Expected 5 per cent.
Variety	2	16	1·0925	0·7788
Manures	16	2	0·6194	>1·4830
Differential response . . .	4	16	0·4121	0·5505

The varietal differences may, therefore, be considered definitely significant, while the effect of manures is negligible.

The mean yield for each variety is shown in Table XI.

TABLE XI.

Mean yield of different varieties of potato.

——	In chattaks per plot	In mds. per *bigha*	In lbs. per acre	Per cent.
Darjeeling	48·2	2·33	560·33	64·0
Patna	86·4	4·18	1004·40	115·0
Deshi	91·5	4·43	1063·69	121·0
Mean	75·4	3·65	876·53	100·0
S. E.	7·96	0·38	92·54	11·0

It is clear that *without irrigation*, Deshi and Patna are likely to give much better results than Darjeeling.

Table XII shows the failure of fertilizers in the absence of irrigation. Ammophos gives slightly better yield, but in view of the magnitude of the standard error no weight can be attached to the result.

TABLE XII.

Mean yield of potato under different manurial treatments.

——	Chattaks per plot	Mds. per *bigha*	Lbs. per acre	Per cent.
No manure	70·6	3·42	820·73	94·0
Potassium nitrate . .	73·8	3·57	857·93	98·0
Ammonium phosphate . . .	81·9	3·96	952·09	109·0
Mean	75·4	3·65	876·53	100·0
S. E.	7·96	0·38	92·54	11·0

Table XIII shows full details of the yield.

TABLE XIII

Mean yield of potatoes in chattaks *per plot of* $\frac{1}{311}$ *acre.*

—	No manure	Pot. nitrate	Ammon. phos.
Darjeeling	51·0	46·0	47·7
Patna	94·0	84·0	81·3
Deshi	66·7	91·3	116·7

S. E.=13·78.

The effect of fertilizers on "Darjeeling" and "Patna" is if anything harmful, while "Deshi" appears to have gained considerable benefit from them.

The division into blocks in this case also does not give any advantage, showing the patchy or non-systematic character of the soil heterogeneity. This accounts for a high residual standard deviation of 23·87 chattaks per plot or expressed as a percentage of the mean yield of 75·41 chattaks per plot, a residual variation of 31·7 per cent. This is considerably higher than the residual variation of 23·8 per cent. found in the case of the paddy experiment.

CONCLUSION

Both the paddy and potato experiments illustrate a simple method of combining varietal and manurial trials in the same field. Fisher has emphasized the importance of such combined experiments. They are not only more efficient in the sense that they can supply replies to two sets of questions—varietal and manurial—at the same time, but are also capable of throwing light on the differential response of varieties to manures which cannot be studied in any other way.

REFERENCES

Mahta, D. N. and Dave, B. B. (1931). *Ind. J. Agric. Sci.* 1, 351.
Robertson, H. F. (1932). *Ind. J. Agric. Sci.* 2, 53.

STATISTICAL NOTES FOR AGRICULTURAL WORKERS

NO. 5.—A NOTE ON THE VARIATION OF PERCENTAGE INFECTION OF WILT DISEASE IN COTTON

BY

P. C. MAHALANOBIS AND SUBHENDU SEKHAR BOSE

(Received for publication on the 18th August 1932)

Mr. G. S. Kulkarni, Special Cotton Mycologist, Dharwar, observed that certain selected strains of cotton, resistant to the wilt disease under field conditions succumb to the same disease under controlled conditions of the laboratory in pot-culture experiments. He is of opinion that resistance to wilt disease under field conditions is chiefly due to the want of favourable conditions for the causal organism of the disease to be pathogenic. To test this assumption a large number of plants of the selected strain "Jayawant" was sown in six replications in three batches in June, July and August, 1931, respectively. As temperature conditions in June and July were believed to be more favourable for wilt production, it was expected that the percentage infection would be greater for the plants sown in June than for those sown in the later months.

The data sent by Mr. Kulkarni covered unequal periods of time for the different replications. Dr. B. B. Mundkar, who was associated with Mr. Kulkarni in this work, has, however, very kindly placed the weekly observations at our disposal. This makes it possible to compare the mortality figures covering 24 weeks after the date of sowing in each case. The relevant data will be found in Table I.

TABLE I

Plot No.	Sowing time	No. of plants sown	No. of wilted plants	Percentage* infection
1	June	1,159	106	9·15
	July	1,097	126	11·49
	August	1,139	129	11·33
2	June	1,125	214	19·02
	July	1,107	185	16·71
	August	1,180	183	12·45

*The percentages were calculated in our Laboratory.

TABLE I—*contd.*

Plot No.	Sowing time	No. of plants sown	No. of wilted plants	Percentage* infection
3	June	1,130	223	19·74
	July	1,100	168	15·27
	August	1,177	109	9·26
4	June	1,117	248	22·22
	July	1,068	115	10·77
	August	1,080	195	18·06
5	June	1,159	316	27·26
	July	1,084	215	19·83
	August	1,107	185	16·80
6	June	1,100	253	23·18
	July	1,042	202	19·38
	August	1,129	173	15·32

From the statistical standpoint, on the available data, it is possible to test whether there are significant differences in percentage infection between plants sown in different months. But, on the given data, it is not possible either to confirm or to reject the hypothesis that the growth of wilt disease in resistant strains in the laboratory is due to the presence of favourable conditions.

The variation in percentage infection may be classified under three heads. Variations due to :

(1) difference in the date of sowing.
(2) differences in the soil-character of the six replications.
(3) Random fluctuations.

The analysis of variance into these three heads is shown in Table II below.

TABLE II

Variance due to	Degrees of freedom	Sum of squares	Mean variance	S. D.
Date of sowing . .	2	124·1949	62·0975	7·88
Soil-differences . .	5	205·4791	41·0958	6·41
Residual . . .	10	112·7506	11·2751	3·35
Soil-differences and residual	15	318·2297	21·2153	4·61
	17	442·4246	26·0249	5·10

$$\text{“}Z\text{”} = \tfrac{1}{2}\log_e \frac{62{\cdot}0975}{11{\cdot}2751} = 0{\cdot}8309$$

5 per cent. point of “Z” (corresponding to $n_1=2$ and $n_2=10$)=·7058.

Since the value of Z is above the 5 per cent. point, the association is real, *i.e.*, the percentage of infection varies significantly with the month of sowing.

The analysis may now be given in detail.

TABLE III

Date of sowing	Mean percentage of infection	Diff. from August	Diff. from July
June	20·10	+6·23	+4·53
July	15·57	+1·70	..
August	13·87	..	—1·70

Standard error on mean difference=1·94
Critical difference for significance—
(5 per cent. level)=4·32, (1 per cent. level)=6·15

* Another salient point that comes out at once from the study of the analysis of variance given in Table II is the marked soil-heterogeneity of the experimental plot used in this experiment. Elimination of soil heterogeneity by the above method reduced the residual variance from a value 21·2153 to value 11·2751.

We therefore conclude the difference in percentage infection between—

(1) June and August is definitely significant.

(2) June and July is on the verge of significance.

(3) June and August is practically insignificant.

The percentage of infection in the different months may also be compared directly in pairs. For this purpose the method originally suggested by "Student" in Biometrika, Volume VI, page 19, may be used with advantage.

TABLE IV.

(1) June and August.

Name of replication	Percentage, June	Infection, August	Difference
1	9·15	11·33	—2·18
2	19·02	12·45	6·57
3	19·74	9·26	10·48
4	22·22	18·06	4·16
5	27·26	16·80	10·46
6	23·18	15·32	7·86
Mean=	20·10	13·87	6·23

Mean difference=6·23

Standard deviation of the differences=4·35

$$\text{"}Z\text{"} = \frac{\text{Mean difference}}{\text{Standard deviation}}$$

$$= \frac{6{\cdot}23}{4{\cdot}35} = 1{\cdot}43 \text{ approximately}$$

Using Biometrika Table XXV (page 36)*, we find that for $n=6$, the probability that the mean difference will not exceed (in algebraic sense) zero by more than 1·43 times the standard deviations of the sample is "P"=·9879, *i.e.*, the probability of the difference being a real one exceeds 98 per cent. The difference may, therefore, be considered definitely significant.

* Fisher's t-table (Table IV) which is based on Student's Table may also be used.

TABLE V.

(2) *June and July.*

Name of replication	Percentage, June	Infection, July	Difference
1	9·15	11·49	—2·33
2	19·02	16·71	2·31
3	19·74	15·71	4·47
4	22·22	10·77	11·45
5	27·26	19·83	7·43
6	23·18	19·38	3·80
Mean=	20·10	15·57	4·53

Mean difference=4·53
Standard deviation of the differences=4·26

"Z"$=\frac{4\cdot53}{4\cdot26}=1\cdot06$, $P=0\cdot9617$

That is, the probability that the mean difference will not exceed zero by more than 1·06 times the standard deviation of the sample is "P"-0·9617. Thus the difference is on the verge of being considered significant.

TABLE VI.

(3) *July and August.*

Name of replication	Percentage, July	Infection, August	Difference
1	11·49	11·33	0·15
2	16·71	12·45	4·26
3	15·27	9·26	6·01
4	10·77	18·06	—7·29
5	19·83	16·80	3·03
6	19·38	15·32	4·06
Mean=	15·57	13·87	1·70

Mean difference=1·70
Standard deviation of the differences=4·39

"Z"$=\frac{1\cdot70}{4\cdot39}=0\cdot39$

"P"$=0\cdot7879$

That is, the probability that the mean difference will not exceed zero by more than ·39 times the standard deviation of the sample, is "P"=0·7879. As the odds are roughly 4 to 1, the difference cannot be considered significant.

The direct comparison thus leads to the same results as those given by the analysis of variance, namely, the percentage infection of plants sown in June is significantly greater than the percentage infection of plants sown in July and August respectively, while the percentage infections of plants sown in July and August do not appear to be significantly different.

The above note was prepared with the help of a grant from the Imperial Council of Agricultural Research.

ABSTRACTS

Interaction between ammonia and soils as a new method of determining the state of saturation and pH values of soils*. AMAR NATH PURI. (*Soil Science* **33**, No. 5, May, 1932).

This is a continuation of the author's previous work in which it was shown that the interaction between soil and ammonia represented the neutralization of acidoid by a base. The object of this investigation was to explore the possibilities of this reaction for characterising the state of saturation of soil.

Since pH values of natural soils represent single points on their titration curves, it is reasonable to suppose that the difference between the amount of ammonia taken up by a natural soil and the same soil completely unsaturated, will be a measure of the state of saturation of that soil and consequently will be correlated with its pH value. The amount of ammonia that a natural soil can take up is determined by keeping a weighed amount over normal ammonia in a vacuum desiccator for two days followed by desiccation over 90 per cent. H_2SO_4 for two days. The ammonia retained by the soil is then determined by distillation with lime in the usual way. The maximum saturation capacity is determined exactly as above after treating the soil exhaustively with 0·2*N* HCl.

The state of saturation of the soil (V) is calculated from the formula :—

$$(V) = \frac{100\ (T-S)}{T}$$

Where S and T are the amounts of ammonia taken up by the natural and acid-treated soil respectively, all quantities being expressed in equivalents.

pH value measurements.

Fifty-seven soils from various parts of India were examined for their reaction by the following methods :—

1. Hydrogen electrode—1 : 5 soil-water ratio, 2 hours' shaking (H).
2. Quinhydrone—1 : 5 soil-water ratio, 2 hours' shaking (Q).
3. Quinhydrone—1 : 1 soil-water ratio, 2 hours' shaking (Q′).
4. Quinhydrone—1 : 1 soil-water ratio, 2 hours' shaking and leaving overnight (Q″)
5. Antimony electrode—1 : 1 soil-water ratio, 2 hours' shaking and leaving overnight (Sb).
6. Colorimetric method—1 : 5 soil-water ratio, occasional stirring, and leaving overnight (C). Clear solution obtained by filtering through a Puri percolating cylinder.

The relation between (V) and various sets of pH values is brought out best by working the correlation coefficients between them. These are given below :—

(V) and (H)	=	0·897
(V) and (Sb)	=	0·891
(V) and (Q′)	=	0·889
(V) and (C)	=	0·938

* This work was carried to completion by the help of a grant from the Imperial Council of Agricultural Research, and the writer takes this opportunity of recording his indebtedness to the Council. Acknowledgments are also due to the Department of Agriculture, Punjab, for the loan of apparatus and other facilities for work.

It will be seen that the correlations are highly significant. The following empirical formulas can be used for calculating pH values from (V) values :—

1. pH = 0·0274 (V) + 5·4 for (V) values below 45 per cent.
2. pH = 0·0319 (V) + 5·89 for (V) values between 45 and 81 per cent.
3. pH = 0·0319 (V) + 7·11 for (V) values above 81 per cent.

Some very interesting facts emerge from a closer study of the (V) values for various soils. It is found that all soils having (V) values less than 33·3 per cent. respond to lime, the calculated pH value for which is about 7. The neutral point or pH 7, therefore, has a more fundamental significance than has been hitherto supposed. Most of the good agricultural soils have (V) values between 50 and 70 per cent. Soils having (V) values above 70 per cent. invariably contain exchangeable sodium and above 80 per cent. are all barren alkali soils. All soils having (V) values less than 40 per cent. are from humid regions where the rainfall is plentiful, and those with above 70 per cent. (V) values are from arid regions where the rainfall is scarce. (*Author's abstract*)

A new type of hydrometer for the mechanical analysis of soils.* AMAR NATH PURI. (*Soil Science* 33, pp. 241-248).

The successful working of the hydrometer method for the mechanical analysis of soils rests on the principle that direct determinations of the variations with time of the density of suspension at a definite distance from the surface in a sedimenting column, would provide data similar in type to those obtained in the pipette method of mechanical analysis.

The new hydrometer consists of a wide, but short bulb (8-10 cm. long) and a thin long stem (about 0·4 cm. in diameter, and 60-70 cm. long). It therefore records density changes in a column 8-10 cm. long and situated at a distance of 50-60 cm. from the top. The settling depth is counted from the top of the sedimenting column to the mean position of the middle point of the hydrometer bulb. A modification in the hydrometer technique is the arrangement for taking readings. The ordinary method of inserting a paper scale in the stem, is not very accurate, and an error of a few millimeters in the readings might be easily made, as a result of the water surface not providing a sharp line of reference. In this case the tip of an ordinary pin attached to a thin brass cap fitting on to the top of the hydrometer stem, forms the reference point, and readings are taken against the graduations on a burette tube, held in a special clamp. The mouth of the burette tube is closed with a brass cap having a hole in the middle through which the hydrometer stem is passed. The edges of the hole are sharpened to prevent friction that might hinder the free movement of the hydrometer. All that is necessary is to tap the burette tube gently before taking a reading. This brass cap has also a pin attached to it which is required for fixing the position of the burette tube with respect to the water surface. The tube is gradually lowered till the pin point just makes contact with the water surface. The burette tube is held in a brass clamp, which keeps it in position, at the same time allowing it to be pushed up or down. The brass clamps are fixed to an iron rod attached to an iron cap that can cover the top of the sedimenting cylinder, and has a hole in the middle to allow the free movement

* This work was carried out by the help of a grant from the Imperial Council of Agricultural Research, and the writer takes this opportunity of recording his indebtedness to the Council Acknowledgments are also due to the Department of Agriculture, Punjab, for the loan of apparatus and other facilities for work.

of the burette tube. This simple device enables one to take hydrometer readings within a fraction of a millimeter, and does away with the necessity of having a paper scale inside the stem. To avoid parallax the graduations on the burette tube should be all round the circumference as in the Charlottenburg type.

Thirteen soils of varied mechanical composition were used for comparing the hydrometer and the standard pipette method. The results discussed in the original paper showed a very good agreement between the two methods.

Half a dozen hydrometers can be conveniently handled for routine work and should prove the quickest means of mechanical analysis, giving directly the summation percentage curves for soils; the results being directly comparable to the pipette method. (*Author's abstract*)

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